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Research Article

Isolation and Characterization of *Saccharomyces cerevisiae* from Ethiopian Mustard (*Brassica carinata*)

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Abstract

Yeast is one of the major fermenting agents in many fermentation processes. There are different types of yeasts with varying fermentation efficiency. Therefore, searching of best yeast strain from different sources is an important strategy for industrial application and other commercial activities. The objective of this study was to isolate best strains of yeast (*Saccharomyces cerevisiae*) from Ethiopian food Sinafich. To achieve this objective, *Brassica carinata* (locally, Gomenzer) were purchased from two sites of Southern Tigray (Mekelle and Alamata). The samples were washed, dried and grinded in to fine powder. Serial dilutions were made up to the factor of 10^{-8} by pouring 1g of the fine powder into 9 mL sterile distilled water. Yeast was cultivated and isolated by spread plating using 100 µL of the aliquots on Malt Extract Agar (MEA) and Yeast Peptone Glucose (YPG) media. The Isolates were further purified and characterized using morphological and biochemical characterizations. Additionally, the fermentation efficiency of the isolates was tested using dough fermentation test. According to the result of this investigation, two best *Saccharomyces cerevisiae* strains were identified from Ethiopian local food Sincafich (Ethiopian Mustard) with better fermentation efficiency and important features such as ethanol tolerance and temperature resistance ability.

Keywords: Fermentation, *Saccharomyces cerevisiae,* Sinafich, *Brassica carinata,* ethanol tolerance.

Introduction

Fermentation is the most important factor in alcohol and food industry. The quality and the rate of production are always determined by the type of yeast they exploit. As a result, various types of yeasts are used for making foods and alcohol production. Yeasts obtain most of their carbon sources by utilizing hexose sugars, such as glucose and fructose either aerobically or in anaerobic condition with varying fermentation efficiency (Barnett, 1975). The utilization of isolated strains of *Saccharomyces cerevisiae* is an important strategy for keeping the quality and assuring the reproducibility of the product features. The utilization of strains isolated from specific regions could be even more interesting because of their high adaptation to their own climatic conditions (Thais *et al.,* 2006). Recently, many investigations have been conducted to explore sources of yeast to seek potent species that can utilize a wide range of substrates for its industrial application. Some locally available food crops and different fermented food were analyzed for isolation and subsequent characterization of yeast isolates which may further be utilized in alcohol and food production (Ebabhi *et al.,* 2013).

In Ethiopia there are different local foods prepared through fermentation process which can be used as best sources of yeast. However, most of the Ethiopian local foods are not investigated for their yeast composition and its fermentation efficiency. Among these, Ethiopian mustard (Sinafich) is one of the most common food type prepared from an indigenous plant *Brassica carinata* (Brassicaceae). The food is commonly used to eat fried meat and other types of food together with pepper. It has pungent smell and powerful test which is prepared through local fermentation process. Therefore this study was intended to isolate and characterize *Saccharomyces cerevisiae* from Ethiopian mustard (locally, Gomenzer) and investigate its fermentation efficiency relative to the conventional *Saccharomyces cerevisiae* strains used in alcohol and food factories.

Materials and methods

Sample collection: Ethiopian mustard was randomly purchased from two districts of southern Tigray region, Alamata and Mekelle. The samples were washed, dried, grinded to fine powder and sieved.

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The fine powder was kept in dried environment at room temperature for further work and used as source of organism.

Isolation and identification of microorganisms: Ten gram of the fine powder was completely mixed with 90 mL of distilled water for 72 h at 28±2°C. About 1 mL of the mixture transferred to 9 mL of distilled water to prepare serial dilutions up to the factor of 10^{-8}. An aliquot of 100 µL was plated on Malt Extract Agar (MEA) plates (5 g/100 mL) using spread plate technique described by Akeredolu *et al*. (2013) and incubated for 48 h at 28±2°C. Moreover, identification was done by culturing the yeast on a specific identification medium in which the consumption of the medium indicates the genus and species of yeast according to the work done by Harrigan and McCance (1976). Identification of yeasts up to species level was carried out based on the standards of cultural, morphological and physiological/biochemical tests. The isolates were designated as AMS (A-Alamata area, M- Mekelle University Microbiological lab, S-Strain and the number refers to the number of strain) and MMS (M-Mekellearea, M- Mekelle University Microbiological lab, S-Strain and the number refers to the number of strain).

Subculture technique: Isolates were sub-cultured on MEA to check the purity and incubated at 28±2°C for 48 h. The shape, color, edge, opacity, elevation, surface and consistency of the colonies were recorded. The strains were stained using methylene blue and viewed under a high power microscope (100× magnification). Color, texture and other features were observed on the colonies (Barnett *et al.,* 2000). Biochemical tests of the selected yeast isolates were carried out by fermentation of different carbon sources using the modified method of Olutiola *et al.* (2000). The identities of the isolates were confirmed by comparing the characteristics with those of known taxa using the schemes of Rhode and Hartmann (1980).

Stress exclusion test: Stress exclusion test was done by culturing the isolates on YPG medium at 37°C for 72 h as described by Thais *et al.* (2006). The isolates were plated on YPG medium at 37°C for 72 h. The grown colonies were plated on YPG supplemented with 80 mL/L ethanol and incubated at 30°C for 72 h. The colonies were then plated on YP supplemented with 200 g/L glucose and grown at the same conditions. After incubation, the colonies were plated in medium Yeast Peptone (YP) supplemented with 200 g/L sucrose and 80 mL/L ethanol and incubated at same conditions.

Ethanol tolerance test: The ethanol tolerance ability of isolates were tested by inoculating a loop full of the colonies in 10 mL of liquid YPG supplemented with 100, 130 and 150 mL/L ethanol and incubating at 30°C for 72 h (Thais *et al.,* 2006).

Temperature tolerance test: Temperature adaptability of isolates were tested by spread plating of isolates on YPG media and incubating at 25, 30, 37 and 45°C (Thais *et al.*, 2006).

Flocculation test: Flocculation ability of the isolates were done by inoculating loop full of isolates on 10 mL of liquid YPG media and incubating at 30°C for 72 h (Thais *et al.,* 2006).

Fermentation efficiency of yeast isolates: The fermentation efficiency of the isolated strains and the commercial yeasts were compared in bread bakery by making 300 g of wheat flour dough using 13 g of commercial yeast and 13 g (wet weight) of isolates were tested according to the standard set by Aboaba and Obakolor (2010) with some modifications. Dough was prepared using the conventional dough making process of Ethiopia using bare hand. Finally, a bread loaf was baked at 180°C for 30 min using oven.

Results and discussion

Based on the colony morphology and microscopic observation, a total of 2 isolates were obtained from the samples collected around Alamata and Mekelle area. The colony morphology showed the characteristics of *Sacharomyces cerevisiae* which is similar with the study of Abosede *et al.* (2013) (Table 1). Carbon source utilization and fermentation ability of the isolates were tested using different carbon sources. All the isolates utilized and fermented glucose, sucrose, lactose and galactose as a carbon source except maltose and dextrose which is similar with the report of Margaret *et al.* (2013). Among the isolates AMS1 and MMS1 showed the fermentation and morphological characteristics of *Saccharomyces cerevisiae* and they were identified as *Saccharomyces cerevisiae* strains (Table 2; Fig. 1).

In some industries, fermentation process will be conducted in the presence of high amount of ethanol and higher level of temperatures. Therefore, best yeast strains with special features such as; strains that withstand ethanol and temperature stress are required (Bauer and Pretorius, 2000). For that reason, an environmental adaptation ability of the isolates for various conditions was checked by exposing to several stress conditions, such as osmotic and ethanol stress as described by Querol *et al.* (2003). The result of the present study indicated that all of the isolates adapted to extreme conditions and they were able to grow in media containing 200 g/L Sucrose and 80 mL/L ethanol which are in line with the result of Pataro *et al.* (2000).

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Table 1. Colony morphology of the isolates.

Isolates	AMS1	MMS1
Organism	*Saccharomyces cerevisiae*	*Saccharomyces cerevisiae*
Pigmentation	White, creamy	White, creamy
Colony morphology	Oblique	Oblique

Table 2. Biochemical characterization of the Isolates.

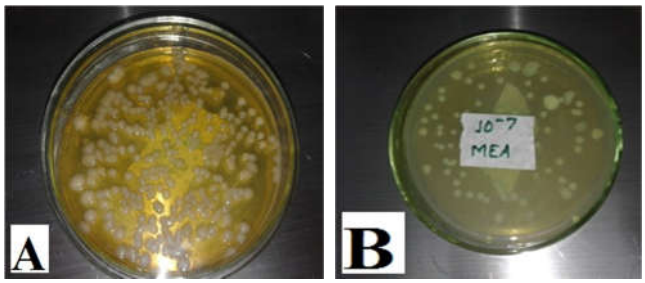
Biochemical characterization	Isolates	
	AMS1	MMS1
Gram reaction	+	+
Sucrose	+	+
Glucose	+	+
Lactose	+	+
Galactose	+	+
Maltose	-	-
Xylulose	-	-
Fructose	+	+
Dextrose	-	-
Urease	-	-

(+) Positive and (-) Negative

Table 3. Ethanol and temperature tolerance.

Isolate	Stress exclusion and temperature tolerance	
	AMS1	MMS1
YPG + 8% Ethanol	+	+
Sucrose + 8% Ethanol	+	+
Temperature (37°C)	+	+

Fig. 1. A) Colony morphology of isolates on YPG media, B) Colony morphology of isolates on MEA media.



Additionally, when these colonies were plated on YPG medium supplemented with 80 mL/L ethanol, all of the isolates were able to grow and they are stress resistant. Moreover, the temperature tolerance test also showed that the yeast isolates were able to grow at maximum temperature of 37°C (Table 3). The nitrogen source assimilation ability of the isolates was tested using potassium nitrate, sodium nitrate and lysine (Thais *et al.*, 2006). Accordingly, all of the colonies from each sample did not grow using potassium nitrate, sodium nitrite and lysine.

This result is also similar with the work of Vaughan-Martini and Mrtini (1993) and Sanni and Lonner (1993). Flocculation test is an important feature which enables us to separate the yeast cells at the end of the fermentation without additional filtration or centrifugation steps. And also, it is important to utilize yeasts on fermentation processes as immobilized cells (Stratford, 1992). Therefore, all the isolates were subjected to flocculation test. However, the result of the present study showed that all of the isolates were flocculation positive. The yeast isolates were tested for their hydrogen sulfide production ability since an elevated hydrogen sulfide production is not desirable characteristic for wine production (Ribeiroi, 1999). The result of this test showed that four of the isolates were identified as non-hydrogen sulfide producing isolates. The dough fermentation ability of the isolates was checked by comparing it with the commercial yeast strains. For this purpose, the dough fermented isolates and the commercial yeast represented by a code were randomly given to 10 individuals to select the best fermented dough. As a result, 8 (80%) individuals selected the dough fermented by the two isolates AMS1 and MMS1, as their priority choice followed by the commercial one.

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Additionally, sample breads baked from all the fermented dough and the breads texture and flavor was checked by the examiners. Similarly, all the examiners selected the bread processed by the two isolates as very good bread followed by the commercial yeast processed breads.

Conclusion

Based on the findings of the present study, best *Saccharomyces cerevisiae* strains were isolated from Ethiopian local food Sincafich (Ethiopian Mustard) with better fermentation ability and very important features such as ethanol tolerance and temperature resistance. These isolates can be substituted for the commercial yeast strains used in different factories and local food fermentation process. However, this study must be supported with molecular characterization and their performance in factories in near future.

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