ISSN: 2581-7566
Volume 1: 1
J Appl Microb Res 2018

Journal of Applied Microbiological Research

Characterization of Yeast Species Isolated from Local Fruits used for Bakery Industrial Application

Tsegaye Z[1*]
Tefera G[1]
Gizaw B[1]
Abatenh E[1]

[1]*Department of Microbiology, Ethiopian Biodiversity Institute, Ethiopia*

Abstract

This study focused on characterization of the Yeast species isolated from local fruits. A total of nine yeasts were characterized for its temperature tolerance, ethanol tolerance, and carbohydrate fermentation, hydrogen sulfide production and leavening ability. Results showed that the yeast species namely *S. cerevisae, S. blourdeous, Z. fermentatii, C. sorboxylosa*, C*. apicola and I. orientalis* were tolerate different ranges of temperatures up to 37^{0}c and concentration of alcohols up to 14%. Only *S. cerevisae* was tolerating 15% alcohol and grow well at 45^{o}c and ferment dextrose, sucrose and fructose. The leavening ability of the identified yeast species were examined by fermenting flour dough. Fermentation test was carried out at room temperature and 30°C for 12 hours. *S. cerevisae, S. blourdeous, Z. fermentati, C. sorboxylosa* and commercial yeast, were able to leaven the highest specific volume of flour dough 230/210cm, 210/191cm, 189/193cm, 184/200cm/g and 180 /182cm respectively at room temperature and 30^{o}c for 12 hours. Thus, wild yeast species namely *S. cerevisae, S. blourdeous* and *Z. fermentati* showed much superior on flour dough leavening ability compared to commercial yeast. Thus, indicates that the local fruit could be a potential source of indigenous yeast species for leavening agent.

Key words: Bakery; Fermentation; Leavening; Yeast.

Background

Leavening agents either chemical or biological are important in raising flour dough. Biological leavening agents are microorganisms that can produce carbon dioxide from the utilization of sugar [1]. Yeast plays an important role in various fermentation processes including baking and brewing. In brewing, the alcohol released by the fungus during fermentation is important while carbon dioxide is of utmost need for rising of flour dough, maturation and development of fermentation flavour [2].

Fermentation of sugars by yeast is the oldest application in the making of bread, beer and wine. The Babylonians (6000BC) and Egyptians (5000BC) have left written accounts of their production of beer, wines and bread, where all of them warranted the use of yeast [3]. Yeast especially *Saccharomyces cerevisiae* is known as sugar-eating fungus and can be found naturally from the surrounding. According to Kurtzman and Fell [4], fruits, vegetables, drinks and other agricultural products are very important microhabitats for several of yeast species. A succession of yeast populations in such products involves in a variety of biochemical processes carried out by yeast to utilize simple sugars present in the agricultural products.

Baker's yeast refers to the strains of *Saccharomyces cerevisiae* used in the bakery industry [5]. The strains are different from the

Article Information

Article Type: Research
Article Number: JAMBR104
Received Date: 16 November, 2017
Accepted Date: 18 Januay, 2018
Published Date: 25 Januay, 2018

***Corresponding author:** Dr. Tsegaye Z, Department of Microbiology, Ethiopian biodiversity institute, Addis Ababa, Ethiopia, Tel no: +251 11 661 2244; E-mail: zerihuntsegaye1970(at)gmail.com

Citation: Tsegaye Z, Tefera G, Gizaw B, Abatenh E (2018) Characterization of Yeast Species Isolated from Local Fruits used for Bakery Industrial Application. J Appl Microb Res Vol: 1, Issu: 1 (21-26).

laboratory strains in their DNA content and chromosomal polymorphism; most of the industrial strains are aneuploids [6]. It required having several characteristics such as high leavening ability, osmo-tolerance, freeze tolerance, chemical tolerance, melibiose utilization, good storage ability, and non-agglomeration [7]. Although some of these characteristics are not necessarily required in some methods for bread making, a high leavening ability is the most important characteristic to produce bread of good quality and to save time in bread making.

Yeast especially S. cerevisiae strains have been selected for decades for their dough-leavening characteristics. The yeast produces carbon dioxide that results in dough leavening and contributes to the flavor and crumb structure of bread [8]. This strain of yeast is very robust and capable of fermenting dough to rise. According to Romano et al. [9], S. cerevisiae can ferment all sugars present in the dough, for example, glucose, fructose, sucrose and maltose with 8 times faster than *P. membranificiens* which can only ferment glucose [10]. As a predominant species it is well known that *S. cerevisiae* plays an important role as a leavening agent in bread making. The leavening step is essential in the fermentation of dough. It does not only induce and increase the volume of dough through gas incorporation but helps creating the desired flavor and texture [11]. Burrows [12] listed four functions of yeast in bread-making: 1) to increase dough volume by evolution of CO_2 during fermentation of the available carbohydrates in the flour, 2) to develop structure and texture in the dough by the stretching due to expansion of gas bubbles, 3) to improve flavor and 4) to add some nutritive values of bread.

Baker's yeast currently used in Ethiopian bakery industries mostly imported from foreign countries such as Australia (Mauripan), France (Saf-instant), Canada (Fermipan) and Turkey (Gold Pakmaya) and are costly. The presence of yeasts from local fruits is yet to be exploited, especially in bakery products as a leavening agent. Up to date there is no report on the use of yeast isolated from Ethiopian local fruits that has potential as a leavening agent in baking industries. Thus, the present study was conducted to characterize wilds yeast species identified from local fruits used for bakery industries. In addition, we also performed brief physiological tests to have better understanding of the yeasts behavior in bread making.

Methods and Materials

Sample collection

The yeast cultures were collected from the Microbial Biodiversity Directorate Gene Bank, Ethiopia Biodiversity Institute. Pure cultures of the identified yeasts were maintained on yeast extract peptone dextrose agar (YPDA) plates.

Temperature tolerance test

The ability of the yeast to grow at higher temperatures was characterized by plating the yeast isolates onto Yeast Peptone dextrose agar medium and was incubated at 5 different temperatures (25, 30, 37and 45°C) for 72 hours [13].

Ethanol tolerance test

The ability of the yeast species to grow in higher ethanol concentrations medium were characterized by growing them in Yeast peptone dextrose broth medium containing 5 different concentrations of ethanol, 2%, 6%, 10%, 14 and 16% (v/v), respectively and incubated at 30°C for 72 hours [13].

Fermentative capacity test

Fermentative capacity of the yeast species identified from local fruits were carried out using (2ml) Yeast Fermentation Broth base with Durham tube for charactering its carbohydrate fermentation. Yeast fermentation broth media was used for characterization of yeasts based on fermentation of specific carbohydrates of fermentation pattern. The carbohydrates used were; glucose (dextrose), fructose, maltose, sucrose, lactose and bromothymol blue). Yeast fermentation broth was used modified media developed by Wickerham for determination of carbohydrate fermentation by yeasts for fermented carbohydrates by yeasts, the color of the medium changed from blue to yellow due to the formation of acids and gas produced. Yeast species identified from local fruits that has good fermentation capacity that they may be used in bakery industry [14].

Hydrogen sulfide production test

Bacto Bismuth Sulfite Agar Dehydrated - BSA (Difco) was used for the selection of the H_2S producing yeast strains. The non-sulfide producing strains had white colonies, while the H_2S producers presented various colony colors that ranged from light brown to black, depending upon the intensity of the production. 20g of BSA was suspended in to 500ml of distilled water.

Heat gently with frequent agitation until the medium just begins to boil and simmer for 30 seconds to dissolve the agar. Cool to 50-55°C, mix well to disperse suspension and pour thick plates. Allow the medium to solidify with the dish uncovered. Larger volumes may be prepared if great care is taken and adequate head space provided. Dry the plates before use but take care to avoid over drying. Correctly prepared plates should have a smooth, cream-like opacity with a pale straw color.

Leavening action of the Yeasts species

Identified yeast species were used to ferment dough to test their leaving ability. Samples of dough were prepared as described by Cauvian and Young [15] using measuring graduated cylinder. Each dough sample contained wheat flour, water and sugar. All the ingredients were properly mixed. Each yeast isolate (10%T) was used to ferment the dough. Baker's yeast (*Saccharomyces cerevisae*) was used separately as positive control yeasts to ferment the dough. Another set of dough formulation that did not contain any yeast sample was prepared as the negative control. The dough samples were left to ferment at room temperature

and 30°c for about 12 hours and. The height of the dough was measured from the graduated surface of the cylinder before and after fermentation and the net increased volume was calculated [16].

Results

Temperature tolerance test

Temperature tolerance capacity of identified yeast species were characterized by plating them onto yeast peptone dextrose agar medium and incubated at different temperatures i.e.25°c, 30°c, 37°c and 45ºC for 72 hours (Table 1). All identified yeast species except *Saccharomyces cerevisae* were grown well at 25°c, 30°c and 37°c. Yeast species were identified from local fruits that survived at high temperature indicate that they may be used as starter culture in bakery industry.

Ethanol tolerance test

Ethanol tolerance test was made by growing of the Identified yeast species in YPG broth containing 5 different concentration of ethanol, 2%, 6%,10%, 14 and 16% (v/v), respectively and incubated at 30ºC for 72 hours (Table 2). The results are presented in Table 2. The yeast strain used in the present study could be classified broadly into three following categories based on their capacity to grow in presence of low or high concentrations of ethanol. (i) Poorly ethanol tolerant yeasts, grown well in to the alcohol concentration between *2* and 6%. (ii) Moderately ethanol tolerant yeast species were grown well in to alcohol concentration between 10 to 14 (iii) Highly ethanol tolerant yeast species were grown well at alcohol concentration from15 up to 16%.

Carbohydrate fermentation test

Carbohydrate fermentation capacity of yeast species isolated from local fruits were conducted using 2 mL Yeast Fermentation Broth (peptone 7.5 g; yeast extract 4.5 g; bromothymol blue) with Durham tube in addition to different carbon sources (1.0 mL sterile carbohydrate solution (dextrose, sucrose, fructose and maltose) and incubated at 25 to 30ºC for 72 h. observe the medium was changed from blue to yellow due to the formation of acids and gas produced [14] if the yeast cells have the ability to ferment the respective sugar. *Saccharomyces cerevisae, Zygosaccharomyces fermentatii* were ferment dextrose, sucrose and fructose, *Saccharomyces cerevisae, Zygosaccharomyces fermentatii, Zygosaccharomyce bisporus* and Kluveromyces *delphensis* were ferment dextrose and sucrose (Table 3). Yeast species that identified from local fruits they may be used as a starter culture in bakery industry.

Hydrogen sulfide production test

Bacto Bismuth Sulfite Agar Dehydrated - BSA (Difco) was used for the selection of the H_2S producing yeast strains. The non-sulfide producing strains had white colonies, while the H_2S producers presented various colony colors that ranged from light brown to black, depending upon the intensity of the production. Five yeast species such as Saccharomyces cerevisae, *Zygosaccharomyces fermentatii Issatechenkia orientalis*, *Zygosaccharomyce bisporus* and *Candida apicola* were strongly produce hydrogen sulfide. Two species namely *Saccharomyces blourdeous* and *Candida sorboxylosa* were did not produce hydrogen sulfide (Table 4). Yeast species that did not produce H_2S under laboratory conditions they may be used as starter culture in bakery industry.

ISSN:

Table 1: Yeasts temperature tolerance test.

Yeast species isolated from local fruits	Temperature			
	25°c	30°c	37°c	45°c
Saccharomyces cerevisae	+	+	+	+
Saccharomyces blourdeous	+	+	+	-
Zygosaccharomyces fermentatii	+	+	+	-
Zygosaccharomyce bisporus	+	+	+	-
Candida apicola	+	+	+	-
Candida sorboxylosa	+	+	+	-
Pichia holistii	+	+		-
Issatechenkia orientalis	+	+	+	-
Kluveromyces delphensis	+	+	+	-
Commercial yeast (*Saccharomyces cerevisiae)*	+	+	+	-

Table 2: Yeasts ethanol tolerance test.

Yeast species isolated from local fruits	Different concentration of alcohol				
	2%	6%	10%	14%	16%
Saccharomyces cerevisae	+	+	+	+	+
Saccharomyces blourdeous	+	+	+	+	-
Zygosaccharomyces fermentatii	+	+	+	+	-
Zygosaccharomyce bisporus	-	-	-	-	-
Candida apicola	+	+	+	+	-
Candida sorboxylosa	+	+	+	+	-
Pichia holistii	-	-	-	-	-
Issatechenkia orientalis	+	+	+	+	-
Kluveromyces delphensis	-	-	-	-	-
Commercial yeast	+	+	+	+	+

Leavening action of the Yeasts species

Leavening capacity of the identified yeast species isolated from local fruit samples were evaluated on dough volume raised in mill liters at 25°c and 30°c for 12 hours duration. Commercial baker's yeast was used separately as positive control yeasts to ferment the dough. The dough samples were left to ferment at room temperature for about 12 hours and the dough in each container was also incubated at 30°C for 12 hours.

The height of the dough was measured from the graduated surface of the cylinder before and after fermentation and the net increased volume was calculated. *Saccharomyces cerevisae, Saccharomyces blourdeous, Zygosaccharomyces fermentatii, Candida sorboxylosa, Zygosaccharomyce bisporus, Kluveromyces delphensis, Pichia holistii, Issatechenkia orientalis* and *Candida apicola* were raise the dough volume of dough up to 230/210cm/g, 220/191 cm/g, 189/193 cm/g, 184/200 cm/g, 160/180 cm/hg, 130/123 cm/g, 120/110 cm/hg, 120/140 cm/g and 120/130 cm/g respectively at 25°c and 30°c for 12 hours. Commercial yeast showed to raise dough volume up to180/182 cm/g at 12hours. Yeast species that identified from local fruits they have excellent leavening capacity may be used as starter culture in bakery industry.

Discussion

During the carbohydrate fermentation process, yeasts produce carbon dioxide, ethanol and other secondary metabolic products which contribute to the formulation of flavor and aroma while the carbon dioxide production reacted as a leavening agent in the dough [10].

Temperature, ethanol and fermentative capacity test were used on the series of yeast's isolates to have a better understanding on yeast behavior. The temperature can affect the fermentation process and the metabolism of yeast. Table 1 illustrates that the growth and inhibition of the yeast species at different growth temperature. Most of the characterized yeast species namely *Saccharomyces blourdeous Zygosaccharomyces fermentatii Zygosaccharomyce bisporus Candida apicola Candida sorboxylosa Pichia holistii Issatechenkia orientalis and Kluveromyces delphensis* were grow well at 37°C except *Saccharomyces cerevisae* (Table 1). *Saccharomyces cerevisae* could tolerate a temperature up to 45°C. The result in this research contradicts the findings of Ma'aruf *et al*., [17], who observed that yeast species isolated from different local fruits could only tolerate a temperature up to 37°C. The ability of yeast to tolerate high temperature suggests that the isolates can resist high heat associated with fermentation process and therefore can be used to accomplish fermentation at wide range of temperature condition. They may also be used in bread making to speed up the baking process, increase carbon dioxide production and formation of flavour and aroma may be enhanced [2].

Yeast species were also characterized on the ability to tolerate different concentrations of alcohol. *Saccharomyces blourdeous, Zygosaccharomyces fermentatii, Zygosaccharomyce bisporus, Candida apicola, Candida sorboxylosa, Pichia holistii Issatechenkia orientalis and Kluveromyces delphensis* identified from local fruits were grown well in a medium containing ethanol concentration from 2% up to 14% (v/v) and failed to grow at 15% (v/v) of ethanol concentration (Table 2). Only *Saccharomyces*

Table 3: Yeasts fermentation capacity test.

Yeast species isolated from local fruits	Different sugars			
	dextrose	sucrose	fructose	lactose
Saccharomyces cerevisae	+	+	+	-
Saccharomyces blourdeous	+	-	-	-
Zygosaccharomyces fermentatii	+	+	+	-
Zygosaccharomyce bisporus	+	+	-	-
Candida apicola	-	+	-	-
Candida sorboxylosa	-	-	+	-
Pichia holistii	-	-		-
Issatechenkia orientalis	-	-	-	-
Kluveromyces delphensis	+	+	-	-
Commercial yeast	+	+	+	-

Table 4: Hydrogen sulfide production test.

Yeast species isolated from local fruits	Hydrogen sulfide (H_2S) production capacity			
	White color No (--)	Light brown color Weak (-)	Dark brown color Strong (++)	Black color very strong (+++)
Saccharomyces cerevisae		+		
Saccharomyces blourdeous	+			
Zygosaccharomyces fermentatii		+		
Zygosaccharomyce bisporus				+
Candida apicola				+
Candida sorboxylosa	+			
Pichia holistii			+	
Issatechenkia orientalis		+		
Kluveromyces delphensis				+
Commercial yeast				+

cerevisae was grown well in medium containing 15% of ethanol concentration indicates that they tolerate ethanol toxicity during fermentation. The results agreed with the findings of [2011], who reported that majority of the yeast species were able to grow in a medium containing 10% and 13% (v/v) of ethanol concentration while only few yeast species could grow in 15% (v/v) ethanol. High concentration of ethanol is reported to be toxic to the yeast by inhibiting the cells growth due to the destruction of the cell membrane [18]. This could have accounted for the inhibiting growth of most of the yeast species at 15% concentration as recorded in this research. A suitable concentration of ethanol is needed in bread making to achieve the preferred flavour [18]. The identified yeast species were also tested on their ability to ferment different sugars namely dextrose, fructose, sucrose and lactose (Table 3). Results show that all strains were able to ferment sugars namely dextrose, sucrose and fructose. Yeast species were ferment different sugars indicates that they may be used as starter culture in bakery industries. This is supported by the fact that yeasts are capable in fermenting sugars especially glucose, sucrose, and fructose (Table 3). The breakdown of sugars will release carbon dioxide that leavens the dough. This indicates that it has enzymes responsible for fermentation of most of the sugars. This could be an important feature for strains used in dough.

Yeast species were also characterized for its ability to produce H_2S using Bismuth Sulfite Agar medium. Majortity of yeast species namely Saccharomyces cerevisae, *Zygosaccharomyces fermentatii Issatechenkia orientalis*, *Zygosaccharomyce bisporus* and *Candida apicola* were strongy produce hydrogen sulfide. While only two species such as *Saccharomyces blourdeous* and *Candida sorboxylosa* were did not produce H_2S (Table 4). H_2S is an undesirable compound associated with an off flavour and unpleasant taste that must be absent in processed foods [19]. Yeasts that showed high production of H_2S are undesirable for bread making because it confers flavour and taste that compromise the quality of the bread [19]. Therefore, the yeast species that did not produce H_2S could be recommended as the best candidate isolates in bread making.

The leavening properties of dough fermented with the various yeast isolates from local fruits. All yeast species namely *Saccharomyces cerevisae, Saccharomyces blourdeous, Zygosaccharomyces fermentatii, Candida sorboxylosa, Zygosaccharomyce bisporus, Kluveromyces delphensis, Pichia holistii* and *Candida apicola* showed good leavening ability giving the specific volume of dough up to 220l/ 210cm/g, 220/191 cm/g, 189/ 193cm/g, 184/200 cm/g, respectively at room temperature and 30°c for 12 hours except for commercial yeast (180/182 cm/g). The results indicate that the ability of the yeast species identified from local fruits is comparable or even better than the commercial yeast to leaven the bread dough and would be considered as the most active yeasts to ferment bread dough compared to other species including commercial yeast strain.

Conclusion

In conclusion, the study reported in this paper indicates that yeast species identified from local fruits are excellent habitat where yeasts with potentials for baking industrial application, particularly yeasts with dough leavening ability as observed in this research. In all the attributes considered, the yeast species namely *Saccharomyces cerevisae, Saccharomyces blourdeous, Zygosaccharomyces fermentatii, Candida sorboxylosa, Zygosaccharomyce bisporus, Kluveromyces delphensis, Pichia holistii* and *Candida apicola* had the overall best performance compared to commercial yeast. It was evidence from these findings that local fruits could be source for baker's yeast which is potentially used as dough leavening agent.

Acknowledgment

First, I would like to thank Ethiopian biodiversity institute, for materials support and microbial biodiversity directorate research team for technical support.

References

1. El Sheikha AF and Montet D (2016) Fermented Foods Artisan Household Technology to Biotechnology Era. Fermented Foods, Part I: Biochemistry and Biotechnology. CRC Press, pp. 1-15.
2. Balarabe MM, Sani SDM, Orukotan AA (2017) Screening of Fermentative Potency of Yeast Isolates from Indigenous Sources for Dough Leavening. Int J Microbiol Biotechnol 2: 12-17.
3. Kevin K (2005) Fungal fermentation systems and products. John Wiley and Sons Ltd, England.
4. Kurtzman CP and Fell JW (1998) The Yeasts: A Taxonomic Study. Elsevier Science, Amsterdam.
5. Phaff HJ, Miller MW, Mrak EM (1978) Industrial uses of yeast. Harvard University Press, Cambridge, MA.
6. Codon AC, Benitez T and Korhola M (1998) Chromosomal polymorphism and adaptation to specific industrial environments of Saccharomyces strains. Appl Microbiol Biotechnol 49: 154-163.
7. Walker GM (1998) Baker's yeast. Wiley and Sons, Chichester.
8. Francisca RG, Pascual S, Jose AP (1999) Engineering baker's yeast: room for improvement. TIBTECH 17: 237-244.
9. Romano P, Angela C, Vincenza S, Rossana R, Cinzia P (2008) Biodiversity of wild strains of *Saccharomyces cerevisiae* as tool to complement and optimize wine quality. World J Microbiol Biotechnol 24: 1797-1802.
10. Umeh SO, Okafor JNC (2016) Isolation, characterization and identification of yeast (*Saccharomyces Cerevisiae*) from three local beverage drinks. International Journal Series in Multidisciplinary Research 2: 44-55.
11. Fleury RY, Bel RR, Juillerat MA (2002) Biogeneration of 2-(1-hydroxyethyl)-4,5-dihydrothiazole as precursor of roasted and popcorn-like aroma for bakery products. Enzyme. J Mol Cata B 20: 473-477.
12. Burrows S (1970) Baker's yeast. Academic press incorporation, London.
13. Thais M, Guimaraes DG, Moriel IP, Machado, Cyntia MT, Fet al. (2006) Isolation and characterization of *Saccharomyces cerevisiae* strains of winery interest. Braz J Pharm Sci 42: 119-126.
14. Warren P and Shadomy L (1991) Yeast fermentation broth base with carbohydrate and Durham tube. Washington DC.
15. Cauvian SP and Young L (1998) Technology of bread making. Blackie Academy and Professional, London.
16. Aboaba OO and Obakpolor EA (2010) The leavening ability of baker's yeast on dough prepared with composite flour (wheat/cassava). Afr J Food Sci 4: 325-329.

17. Ma'aruf, AG, Noroul Asyikeen Z, Sahilah AM and Mohd. Khan A (2011) Leavening Ability of yeast isolated from different local fruits in bakery products. Sains Malaysiana 40: 1413- 1419.

18. Stanley D, Bandara A, Fraser S, Chambers PJ, Stanley GA (2010) The ethanol stress response and ethanol tolerance of *Saccharomyces cerevisiae*. J Appl Microbiol 109: 13-24.

19. Del Castillo Lozano ML, Tache R, Bonnarme P, Landaud S (2007) Evaluation of a quantitative screening method for hydrogen sulfide production by cheese-ripening microorganisms: the first step towards l-cysteine catabolism. J Microbiol Methods 69: 70-77.

Citation: Tsegaye Z, Tefera G, Gizaw B, Abatenh E (2018) Characterization of Yeast Species Isolated from Local Fruits used for Bakery Industrial Application. J Appl Microb Res Vol: 1, Issu: 1 (21-26).

ISSN: 2581-7566