Characterization of Indigenous Yeasts isolated from some Fruits collected from Fruit Vendors

Eshet Lakew (eshetbiot@gmail.com)
Hawassa University

Research Article

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Abstract

Background

Yeasts are the best leavening agents that principally break down sugars of the flour into carbon dioxide and ethanol. Therefore, the present study was carried out to characterize the indigenous yeast isolates recovered from fruits. The indigenous yeast isolates were retrieved from fruits following standard methods.

Results

From a total of 88 yeast isolates, three of them were selected on the basis of not producing H_2S and their high sugar fermentation abilities. Based on colonial, morphological parameters and biochemical tests, the three yeast isolates were identified as members of the genus *Saccharomyces*. The optimum growth pH and temperature values for the three selected yeast isolates were recorded as 5 and 30^{o}C, respectively in Yeast Extract Peptone Dextrose medium. The results indicated that 30% (w/v) D-glucose and 5% (w/v) NaCl concentrations supported optimum growth of the isolates in the same medium. In all cases, the maximum biomass was achieved at 96 hrs of incubation but decreased rapidly afterwards.

Conclusion

Therefore, these three non-H_2S but sufficient CO_2 producers indigenous yeast isolates can be used to bread baking with and serve as a potential leavening agent for the bakery industry and can be used to produce ethanol.

Introduction

Microbes are generally regarded as part of wildlife or undeveloped raw materials of a locality, region or nation. Therefore, the practice of isolating and evaluating local microbial strains for defined or potential commercial attributes is a common practice in industrial microbiology/microbial biotechnology [18].

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

[13]. Yeast especially *Saccharomyces cerevisiae* is known as sugar-eating fungus and can be found naturally from the surrounding. According to [16], 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.

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 flavor [3, 28].

The yeast species that dominates in the formation of wine worldwide is *Saccharomyces cerevisiae*. Selecting a particular strain of this species for fermentation can substantially impact various beverages' flavor and aroma characteristics [4, 5]. These yeast species live in a wide variety of ecological niches. Still, the most prevalent habitat is the fruit surface of mainly grapes and berries. They become actively involved in the decomposition of ripe fruit as they assist in the

fermentation process [25, 2]. Indeed, the indigenous yeasts' effectiveness on the surface of these undamaged grapes relies on their diverse physiological characteristics such as killer phenotype, sugar availability, total acidity, pH, etc. [22].

Saccharomyces cerevisiae, as it is a sugar loving microorganism, it can be isolated from sugar rich sources. Fruits contain high sugar concentration that can be utilized by yeast species and this making their isolation from these sources possible [25].

Leavening agents either chemical or biological are important in rising flour dough. Biological leavening agents including yeasts and lactic acid bacteria have the ability to produce carbon dioxide and other diverse compounds by utilizing sugars [9]. *Saccharomyces cerevisiae* is the most commonly used agent used for bread baking. In bread making, the most important function of baker's yeasts is leavening by producing CO_2 via the alcoholic fermentation of the sugars which increases the dough volume by giving bread its characteristic light and spongy texture [8].

[6] showed that modern baker's yeast strains differ from other industrial yeasts on the features including their maximum growth temperature, moderate tolerance to low pH and high growth rate on glucose.

During the course of dough fermentation, some strains of *Saccharomyces cerevisiae* produce off-flavors [12]. One of such off-flavor compound is hydrogen sulfide [12]. Hence, yeasts those to be used for bread baking should not produce this undesirable compound [12].

The presence of yeasts from local fruits is yet to be exploited, especially in bakery products as a leavening agent and for the production of ethanol.

Hence, this study was aimed at isolating, screening, selecting, identifying indigenous yeasts with excellent fermenting potential from various fruit sources to use as a leavening agent and for the production of bioethanol in the future.

Results

Characterization of yeast isolates

In this study, 88 yeast isolates (13 from avocado, 14 from banana, 11 from grape, 11 from mango, 15 from orange, 14 from papaya, and 10 from pineapple) were isolated, purified and further identified (data not shown).

Characterization of selected yeast isolates

Comparison of cell morphology of the yeast isolates under the microscope indicated that AAUGr5, AAUPi3 and SFI (commercial yeast) were found to have oval shape and AAUOr7 had spherical shape. Yeast isolates, AAUOr7 and AAUGr5 exhibited budding pattern of single, paired and triplet features (Data not shown). Likewise, SFI displayed single and paired budding pattern and isolate AAUPi3 showed only single budding pattern.

Biochemical characteristics of selected yeast isolates and their growth on PDA

The selected yeast isolates and the control were observed growing on all sugars except lactose (Table 1). All the isolates and the control were also exhibited the ability to grow using $(NH4)_2SO4$ as a nitrogen source but not KNO_3 (Table 1).

Table 1 Biochemical characteristics of yeast isolates

Isolate	D-glucose	Fructose	Maltose	galactose	Lactose	Sucrose	KNO3	(NH4)2 SO4	Tentative identity
SFI	+	+	+	+	-	+	-	+	S. cerevisiae
AAUGr5	+	+	+	+	-	+	-	+	Saccharomyces
AAUOr7	+	+	+	+	-	+	-	+	Saccharomyces
AAUPi3	+	+	+	+	-	+	-	+	Saccharomyces

+=The isolate fermented the carbon source and utilized the nitrogen source, - = the isolates didn't ferment the carbon source and didn't utilize the nitrogen source

Production of CO_2 and H_2S

From a total of 88 wild yeast isolates, 6 of the yeast isolates were selected on the basis of production of CO_2 observed within 24 hrs (Table 2). Among the selected 6 yeast isolates that produced higher level of CO_2, isolates AAUMa4, AAUBa2, AAUMa6 and the commercial yeast (SFI) were observed producing H_2S gas. Yeast isolates, AAUGr5, AAUPi3 and AAUOr7 were found not producingH_2S on both Bismuth Sulfite Agar (BSA) and Kligler Iron Agar (KIA) media (Fig. 1a and b). Therefore, AAUGr5, AAUPi3 and AAUOr7 were selected for further analysis.

Table 2 isolates producing more CO_2 from each substrate

Substrates	Total number of purified Yeasts isolates	(%) CO_2 production within 24 hrs		Isolates found the best CO_2 producer
		No.(%) Producers	Non-producers	
Avocado	13	0 (0%)	13 (14.77%)	-
Banana	14	7 (7.95%)	7(7.95%)	AAUBa2
Grape	11	7 (7.95%)	4 (4.55%)	AAUGr5
Mango	11	2 (2.27%)	9 (10.23%)	AAUMa4 & AAUMa6
Orange	15	7 (7.95%)	8(9.09%)	AAUOr7
Papaya	14	0 (0%)	14 (15.90%)	-
Pineapple	10	7 (7.95%)	3 (3.41%)	AAUPi3
Total	88	30 (34. 09%)	58 (65.91 %)	

AAUBa2= banana, AAUGr5=grape, AAUMa4 and AAUMa6= mango, AAUOr7= orange and AAUPi3= pineapple

Effect of Temperature, pH, D-glucose and NaCl

The biomass yield of the isolates ranged from 0.004 to 1.53 OD at different incubation temperature (Fig.2). All yeast isolates observed growing moderately at 25^{o}C and 35^{o}C and at biomass decreased at 42^{o}C. The highest biomass was recorded when all the yeast isolates were grown at 30^{o}C with corresponding values for each isolate: the optimum OD

value was observed at 96 hours of incubation for the isolate AAUPi3 (1.53), AAUOr7 (1.50), AAUGr5 (1.07) and SFI (1.14) (Fig.2). Extending the incubation period did not show increase in biomass at all temperature values (Fig.2).

The yeast isolates were able to grow at different pH values (4, 5 and 6) at 30^{o}C (Fig. 3). The maximum OD readings of the yeast growth at 550 nm was observed at pH 5 for all the yeast isolates with corresponding values for each isolate: AAUPi3 (0.95), AAUOr7 (0.89), and AAUGr5 (0.88) as compared to SFI (0.99) (Fig.3). The SFI (0.99) and AAUPi3 (0.95) were found producing more biomass than the other two. In all cases, the optimum pH value was shown to be at pH 5 for all the yeast isolates (Fig.3).

All the yeast isolates had the highest growth in 30% D-glucose containing yeast extract and peptone 5 g/l medium. Growth of each yeast isolate was found gradually lowering in 40% D-glucose and then further decreased in 50% D-glucose (Table 4). The maximum mean growth of biomass reading for all the yeast isolates were found as 1.81 (AAUPi3) followed by 1.74 (AAUOr7), 1.70 (AAUGr5) and 1.78 (SFI) (Table 4).

Table 4 Effect of D-glucose on the growth of yeast isolates

D-glucose (%)	Growth (OD at 550nm) of the isolates at different D-glucose concentrations			
	AAUPi3	AAUOr7	AAUGr5	SFI
30	1.81±0.03a	1.74±0.05a	1.70±0.04a	1.78±0.01a
40	1.67±0.05b	1.63±0.02b	1.59±0.07ab	1.72±0.01b
50	1.56±0.00c	1.51±0.02c	1.38±0.00b	1.58±0.00c

AAUPi3=pineapple, AAUOr7=orange, AAUGr5=grape and SFI=commercial yeast. Means followed by the same letters within a column are not significantly different ($p<0.05\%$)

All the yeast isolates had the highest biomass in 5% NaCl (w/v) containing yeast extract and peptone (5 g/l) medium but gradually lowered in 10% and then further declined in 15% NaCl (w/v). The maximum mean growth of biomass reading for the yeast isolates was recorded for isolates AAUGr5 (1.38) followed by AAUPi3 (1.02) and AAUOr7 (0.97) compared to SFI (1.73) (Table 5).

Table 5. Effect of NaCl on the growth of yeast isolates under aerobic conditions

NaCl (%)	Growth (OD at 550nm) of the isolates at different NaCl concentrations			
	AAUPi3	AAUOr7	AAUGr5	SFI
5	1.02±0.08a	0.97±0.02a	1.38±0.06a	1.73±0.01a
10	0.90±0.01ab	0.87±0.00b	1.11±0.01b	1.63±0.01b
15	0.80±0.02b	0.72±0.01c	0.94±0.01c	1.12±0.01c

AAUPi3=pineapple, AAUOr7=orange, AAUGr5=grape and SFI=commercial yeast

Means followed by the same letters with in a column are not significantly different ($p<0.05\%$)

Discussion

In this study, 88 yeast isolates from local fermented fruits were isolated and compared with the commercial baker's yeast. The isolates had white and creamy color, ovoid and spherical microscopic shape, 4- ascospores in ascus and capable of budding. These results were in consistent with the previous findings that indicated yeasts with similar features are grossly identified as *Saccharomyces* [23]. The morphological characteristics of the three yeast isolates observed by using microscope (X100 magnifications) showed that the three isolates were unicellular with spherical or oval cell shape, comparable to the commercial yeast. This result is in agreement with [10] who confirmed the characteristics of *Saccharomyces* recovered from local substrates. In this experiment, yeast isolates AAUPi3, AAUOr7 and AAUGr5 were found to be the best gas producer observed in the Durham tube among the 88 yeast isolates.

The results showed that all the yeast isolates including the commercial yeast were able to ferment the six sugars tested and released carbon dioxide gas as observed in Durham tube except lactose. This could be an important indication of invertase activity exhibited by yeast isolates used in this study. Similarly, [26] has reported that *Saccharomyces* which was unable to ferment lactose lacked lactase or β-galactosidase system.

The potent three yeast isolates including the commercial strain grew on $(NH4)_2\ SO_4$ as a sole source of nitrogen. But KNO_3 had inhibitory effect on the growth of the yeast isolates including the commercial control. Thus, addition of nitrogen sources in the form of ammonium salts and aqueous ammonia, is generally needed in order to support the growth of yeast cells [24]. Biomass yields varied considerably and were found to be dependent on the types of the yeast strain and the type of nitrogen source used. If sugars are fermented in the presence of adequate amount of nitrogen, less alcohol is formed because the environment is more favorable for the growth of the yeast.

In this study, some of the yeast isolates produced black color in BSA and KIA media including the commercial yeast strain that significantly compromised bread quality. H_2S is undesirable compound associated with an off-flavor and imparts unpleasant taste in processed foods [21]. Yeasts with elevated production of hydrogen sulfide are undesirable for bread baking because it contributes to undesirable flavor and taste that compromises the quality of the bread [29].

The optimum biomass was observed at 30^oC by all the yeast isolates. The result of this study is in consistent with the findings of other investigators [19] who have obtained maximum cell mass production at 30°C. There was a rapid decrease in cell number for all the yeast isolates after 96 hrs of incubation at all tested temperatures since the synthesis of enzyme is affected by increased temperature beyond 30^oC [15].

The result of this study indicated that pH 5 was found to be the optimum value for the growth of the yeast isolates including the commercial yeast control. In a similar study, *Saccharomyces* was found to grow optimally at pH of 5 [1]. In general, the three yeast isolates (of this study) were found to perform close to the commercial yeast with respect to pH and temperature tolerance.

In this study, the yeast isolates were able to grow in Yeast Extract Peptone Broth containing different D-glucose and NaCl concentrations. Accordingly, the optimum growth was obtained at 30% D-glucose and 5% NaCl. This finding is in agreement with [20] who have demonstrated that most of *Saccharomyces* species isolated from conventional fermentation processes and those were physiologically adapted to extreme conditions. The same workers have indicated that the yeasts were not inhibited by the presence of salt and sugar.

Conclusion

Indigenous yeast can be isolated from different natural substrates mainly in fruits. In this study the three yeast isolates were identified as genus Saccharomyces, in contrast to the control commercial yeast, these yeast isolates on top of producing sufficient amount of CO_2, were non-H_2S producers, which is a highly desirable feature for bread leavening and ethanol production.

Material And Methods

Sampling site and sample collection

A total of fifty-six fruit samples, of which eight each from avocado (*Persea americana*), banana (*Musa acuminate*), grape (*Vitis vinifera*), mango (*Mangifera indica*), orange (*Citrus sinensis L.*), papaya (*Carica papaya*) and pineapple (*Ananascomosus*). All samples were collected from major local markets (Atikilit Tera and Merkato) in Addis Ababa City.

Sample preparation

Fruit samples preparation for yeast isolation was done following the method indicated by [27]. 100g of each fruit sample was separately cut, crushed with mortar in sterile plastic bags. The homogenate of each fruit sample was transferred into sterile beakers along with 50 ml of sterile distilled water. The covered beaker with each fruit homogenate was kept at room temperature for three days to allow fermentation to take place. In this study, active dry baker's yeast (Saf instant) from DSM bakery Ingredients, Holland was used as a standard strain.

Isolation of yeasts

From each separate fermented fruit sample, 1ml was serially diluted in 9 ml sterile peptone water in a test tube. From appropriate dilutions, 0.1 ml of aliquots were spread plated on pre-dried potato dextrose agar (PDA, HIMEDIA) plates that contained chloramphenicol (0.1 g/L). All the plates were incubated at 30^{o}C for 48 hrs.

From each countable plate, ten to twenty colonies were taken and purified on the similar freshly prepared medium. The purified isolates were transferred to PDA slants and preserved at 4^{o}C for further study.

Test of CO_2 and H_2S production of yeast isolates

The level of CO_2 production by each yeast isolate recovered in this study was tested first by inoculating each pure isolate into a test tube that contained 5 ml of Yeast Extract Peptone Dextrose (YEPD) broth with a Durham tube. All the tubes were incubated at 30^{o}C for 24 hrs and those isolates that were producing more amount of CO_2 by displacing the medium in the Durham tube were selected. H_2S production by each purified yeast isolate was verified after having those potential isolates by streaking each isolate on Bismuth Sulfite Agar (BSA) and Kligler Iron Agar (KIA) plates. All plates were incubated at 30^{o}C for 3 days following the procedures of [11]. Isolates that exhibited black color on BSA plates and any blackening of the KIA along the line of inoculation or throughout the butt indicate hydrogen sulfide production.

Characterization of selected yeast isolates

Identification of yeast isolates to genus level was carried out on the basis of standard cultural, morphological and biochemical tests as described by [7]. Induction of ascospore formation and its observation was done following the protocol given by [17] and [14].

Biochemical characterization

The ability of each of selected yeast isolates in utilizing D-glucose, fructose, maltose, galactose, lactose and sucrose as a sole carbon source and production of gas was determined in Durham tubes following the standard methods as indicated in [7]. Briefly, the medium was prepared by adding 10 g of yeast extract, and 10 g of peptone in 1000 ml of distilled water and thoroughly mixing. The pH was adjusted to 5 and the medium was boiled. Bromocresol purple carbohydrate (2%, w/v) fermentation indicator was added to an already boiled broth and dispensed in 5 ml amount into screw capped test tubes containing inversely placed Durham tubes. Both KNO_3 (10 g/l), and $(NH4)_2SO_4$ (10 g/l) were used as nitrogen sources. The solutions were sterilized at 121^{o}C for 15 minutes in separate flasks. The carbohydrate fermentation test was

performed by inoculating yeast cells (6.6 log cfu/ml) of each isolate into separate tubes of 5 ml of yeast extract peptone broth with different sugar and nitrogen sources. The inoculated tubes were incubated at 30^{o}C for 48 hrs. Change of color from violet to yellow due to acid production and accumulation of gas bubbles in inverted Durham tube (CO_2 gas production) was taken as a positive result of sugar fermentation. No color change was taken as a negative result.

Effect of temperature, pH, glucose, and sodium chloride concentrations on growth of yeasts

The growth of each selected yeast isolates at different temperature values (25, 30, 35 and 42^{o}C) was carried out by inoculating each actively growing yeast isolate (log 6.6 cfu/ml) into 50 ml of sterile YEPD broth (pH adjusted to 5 before autoclaving) in separate flasks.

Flasks were incubated at respective temperature values and the growth of each isolate was determined by reading the optical density at 550 nm using a spectrophotometer (6405 UV/Vis, JENWAY, United Kingdom) at intervals of 24, 48, 72, 96 and 120 hrs. To examine the optimum pH for the growth of the three selected yeast isolates, the pH of YEPD broth in separate flasks was adjusted to 4, 5 and 6 using 1N HCl and NaOH. Into each separate flask with a given pH value, each of an actively growing selected yeast isolate (log6.6 cfu/ml) was inoculated. The flasks were incubated at 30^{o}C. The growth of each yeast isolates in a given pH value was followed at 24, 48, 72, 96 and 120 hrs by measuring the optical densities at 550 nm using a spectrophotometer (6405 UV/Vis, JENWAY, United Kingdom).

The ability of each yeast isolates to grow at different D-glucose concentrations (30, 40 and 50%) was conducted in YEPD medium. The pH of the broth medium was adjusted to 5. Similarly, log6.6 cfu/ml of each of an actively growing isolate was inoculated into 50 ml flasks. The flasks were incubated at 30^{o}C. The growth of each isolate was determined after 96 hrs by measuring the optical densities at 550 nm using a spectrophotometer (6405 UV/Vis, JENWAY, United Kingdom).

The growth of the yeast isolates at different NaCl concentrations (5, 10 and 15%) (w/v) in YEPD medium was prepared and the pH of the broth was adjusted to 5. Each actively growing isolate (log6.6 cfu/ml) was inoculated into 50 ml flask and incubated at 30^{o}C. The growth of each isolate was determined after 96 hrs by measuring the optical densities at 550 nm using a spectrophotometer (6405 UV/Vis, JENWAY, United Kingdom).

Data analysis

All data were presented as the average of triplicate experiments with standard deviation. Results were statistically interpreted with one-way analysis of variance (ANOVA) followed by post hoc analysis (Tukey's test) to locate the significant differences. The data for ANOVA were analyzed using SPSS version 20.0 at $p<0.05$% significant level.

Declarations

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Author contribution

EL conceived the study, designed the experiments, performed experiments, analyzed and interpreted results. EL wrote the manuscript, and edited the manuscript. and finally approved the final version of the manuscript.

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Availability of data

Data is available and will be provided up on request

Competing Interest

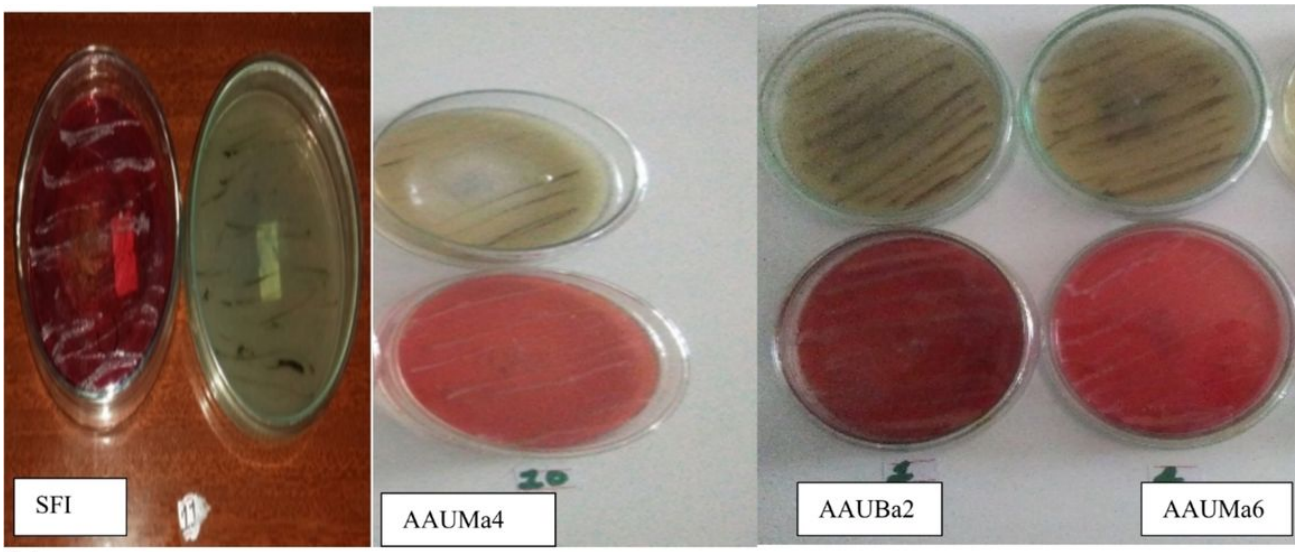
Author declares that there is no conflict of interest in the study.

References

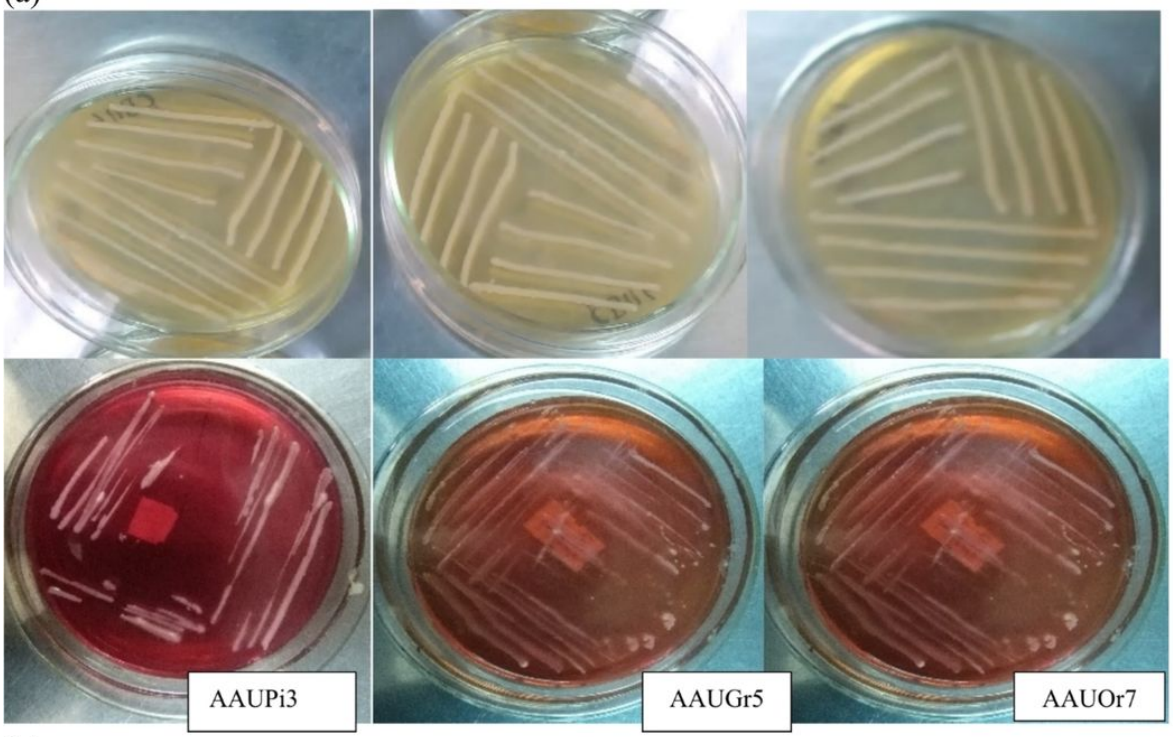
1. Azmuda N, Jahan N, Khan A R. Production and Comparison of Indigenous and Commercial Baker's Yeasts. Bangladesh J. Microbiol. 2006; 2: 89-92.
2. Arias C R, Burns J K, Friedrich L M, Goodrich R M, Parish M E.Yeast Species Associated with Orange Juice: Evaluation of Different Identification Methods. *Appl. Environ. Microbiol*. 2002; 68, 1955-1961.
3. Balarabe MM, Sani SDM, Orukotan AA. Screening of Fermentative Potency of Yeast Isolates from Indigenous Sources for Dough Leavening. Int. J. Microbiol. Biotechnol.2017; 2: 12-17.
4. Bell S J and Henschke P A.Implications of nitrogen nutrition for grapes, fermentation and wine. *Aust. J. Grape Wine Res.* 2005;11, 242-295.
5. Cappello MS, Bleve G, Grieco F, Dellaglio F, Zacheo G. Characterization of Saccharomyces cerevisiae strains isolated from must of grape grown in experimental vineyard. *J. Appl. Microbiol.*2004; 97, 1274-1280.
6. Dawson M P. Classification of industrial strains of Saccharomyces cerevisiae based on physiological characteristics. Master of Applied Science in Food Microbiology Thesis, University of New South Wales, Sydney.1994.
7. De Maristela A V, Luciano G F, Miranda I C, Ana dos N G S, Mauricio X C and Rogelio L B. Isolation of Saccharomyces cerevisiae strains producing higher levels of flavoring compounds for production of "cachaca" The Brazilian sugarcane spirit. *Int. J. Food Microbiol*.2006;108: 51-59.
8. Dunn B, Levine R P and Sherlock G. Microarray karyotyping of commercial wine yeast strains reveal shared, as well as unique, genomic signatures. *BMC Geno*. 2015; 6: 53.
9. El Sheikha A F and Montet D. Fermented Foods- Artisan Household Technology to Biotechnology Era. Fermented Foods, Part I: Biochemistry and Biotechnology. 2016.
10. Greame M, Walker and Nia A W. Introduction to Fungal Physiology. In: Fungi Biology and Application, Pp.1-34, (Kevin, K., John Wiley and Sons, eds), England. 2005.
11. Jiranek V, Langridge Pand Henschke P A. Validation of bismuth containing indicator media for predicting H_2S-producing potential of Saccharomyces cerevisiae under enological conditions. *American J. Enol. Viticu*. 1995; 46: 269-273.
12. Jiranek V A P, Langridge and Henschke P A. Determination of sulfite reductase activity and its response to nitrogen status in a saccharomyces cerevisiae wine yeast. *J. Appl. Bacteriol*. 1996; 81:329-336.
13. Kevin K. Fungal fermentation systems and products. John Wiley and Sons Ltd, England. 2005.
14. Kirsop B E and Kurtzman C P. Living Resources for Biotechnology: YEASTS. Cambridge University Press, Cambridge. 1988.
15. Knox R. The effect of temperature on enzymatic adaptation, growth and drug resistance. Symposium of the Society for General Microbiol. 1955; 3: 184-189.
16. Kurtzman CP and Fell JW. The Yeasts: A Taxonomic Study. Elsevier Science, Amsterdam. 1998.
17. Lodder J. The Yeasts: A Taxonomic study. North Holland Publishing, Amsterdam. 1971.

18. Olabisi Oloruntoba Olowonibi. Isolation and Characterization of Palm Wine Strains of Saccharomyces cerevisiae Potentially Useful as Bakery Yeasts. *Eur. J. Exp. Biol*. 2017; 7:11
19. Parveen M. Isolation, characterization and evaluation of indigenous baker's yeast. MSc. Thesis. Department of Microbiology, University of Dhaka, Dhaka. 1991.
20. Pataro C, Guerra J B, Petrillo-PeixotoM L, Mendonça H LC, Linardi V R and Rosa C A. Yeast communities and genetic polymorphism of Saccharomyces cerevisiae strains associated with artisanal fermentation in Brazil. J. Appl. Microbiol. 2000; 88: 1-9.
21. RiberioC A F and HoriiJ. Potencialidades de linhagens de levedura Saccharomyces cerevisiae para a frementacao do caldo de cana. Sci. Agri. 1999; 56: 255-263.
22. Salari R. Investigation of the Best Saccharomyces cerevisiae Growth Condition. *Electron. Physician*. 2017; 9, 3592-3597.
23. Samuel Sahle and BirhanuAbegaz. The microbiology of baker's yeast fermentation. SINET: Ethiop. J. Sci. 1991;14: 81-92.
24. Sheikh and Berry D R. Biotechnology Letters. 2009;2: 61-66.
25. Spencer J F T and Spencer D M. *Yeasts in Natural and Artificial Habitats*; Springer: Berlin, Germany. 1997.
26. Tarek M E N. Immobilization of recombinant strains of Saccharomyces cerevisiae for the hydrolysis of lactose in salted Dominated cheese whey. *Euro. Food Research and Technol*. 2001; 212: 225-227.
27. Thais M, Guimarães, Danilo G Moriel, Iara P Machado, Cyntia M T, FadelPicheth and Tania M B, Bonfm. Isolation and characterization of Saccharomyces cerevisiae strains of winery interest. *Brazilian J. Pharm. Sci.* 2006; 42: 550-561.
28. Tsegaye Z, Tefera G, Gizaw B, Abatenh E. Characterization of Yeast Species Isolated from Local Fruits used for Bakery Industrial Application. J Appl Microb Res. 2018; 1: 1
29. Vicente M A LG, Fiettol M,Castro A N, dosSantos MX,Coutrim and R L Brandao. Isolation of Saccharomyces cerevisiae strains producing higher levels of flavoring compounds for production of Cachaca the Brazilian Sugar Cane Spirit. *Int. J. Food Microbiol*. 2006; 108: 51-59.

Figures



(a)



(b)

Figure 1

Observation of H_2S gas production by cultures on BSA (upper) and KIA media (lower). (a) SFI= commercial yeast, AAUMa4 and AAUMa6 = mango, and AAUBa2= banana; (b) AAUPi3 = pineapple, AAUGr5=grape and AAUOr7= orange; a= H_2S producers; b= Non H_2S producers

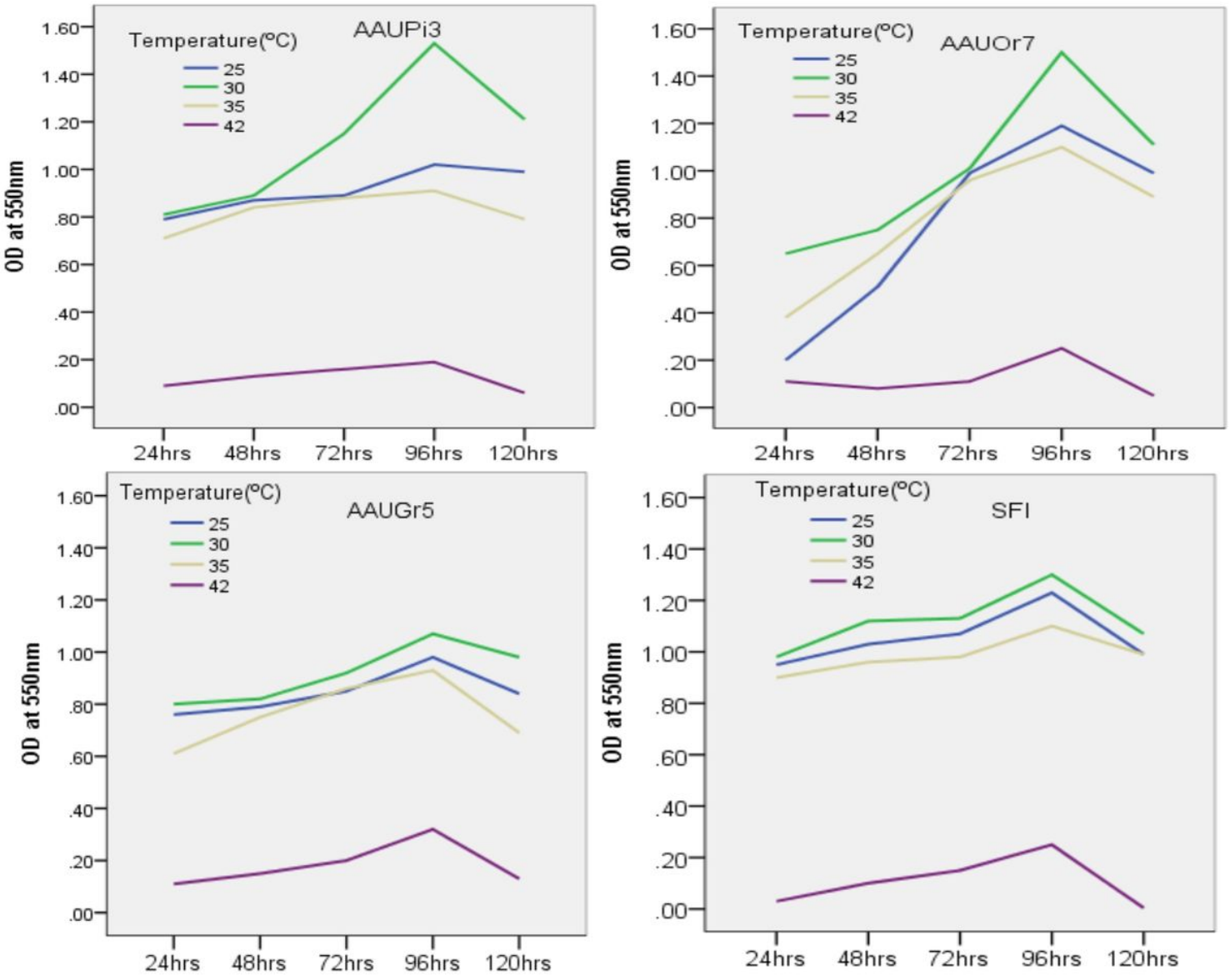


Figure 2

Effect of temperature on the growth of wild yeasts in YEPD medium incubated for 24, 48, 72, 96 &120 hrs.

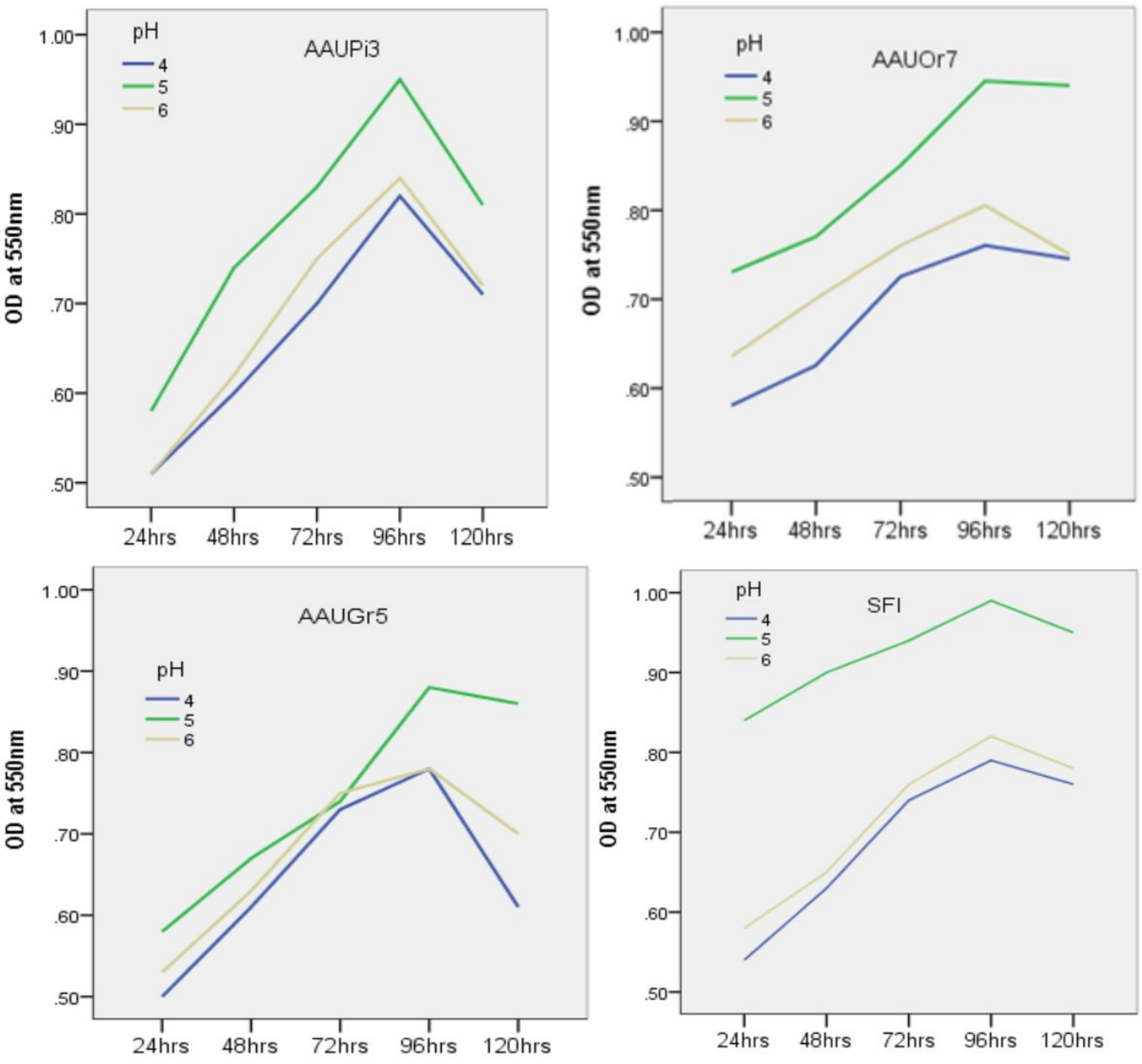


Figure 3

Effect of pH on the growth of yeasts in YEPD after incubation for 24, 48, 72, 96 &120 hrs