College of Natural and Computational Sciences

Department of Microbial, Cellular and Molecular Biology

Characterization of Indigenous Wild Yeasts and Evaluation of their Bread Leavening Capacity

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A Thesis Submitted to the School of Graduate Studies of Addis Ababa University in Partial Fulfillment of the Requirement for the Degree of Masters of Science in Applied Microbiology

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ABBREVIATION AND ACRONYMS

^0C	Degree Centigrade
Cm^3	Centimeter cube
%	Percentage
α	Alpha
CO_2	Carbon dioxide
H_2S	Hydrogen sulphide
ANOVA	Analaysis of Variance
ATP	Adenosine triphosphate
bp	Base pair
et al.	And other people
ETB	Ethiopian Birr
g	Gram
Hrs	Hours
ITS	Internal transcribed spacer
Kg	Kilogram
L	Liter
Min	Minute
Ml	Milliliter
NADH	Nicotinamide adenine dinucleotide
Nm	Nano meter
No.	Number
OD	Optical density
pH	Negative logarithm of hydrogen ion concentration
RBCA	Rose Bengal chloroamphinicol agar
rpm	Revolution per minute
rDNA	Ribosomal Deoxynucleic Acid
rRNA	Ribosomal Ribonucleic Acid
µl	Micro liter
µ mol	Micro mole
YPDA	Yeast extract peptone dextrose agar

ABSTRACT

Ethiopia is a developing country with a high baker's yeast demand for bread making and beverage industries. However, there is no baker's yeast producing plant in the country. Therefore, the objective of this study was to assess bread leavening potential of wild yeasts from *teff* and wheat dough, *shamita, tej, tella*, molasses and honey samples, following standard methods. A total of 180 wild yeast isolates were, out of these isolates 23 isolates with high biomass yield (1.7 to 2.6 of 600 nm OD reading) and the efficient gas (CO_2) producers in durum tube within 12 to 24 hours were selected for further analysis. Morphological, biochemical and molecular features were used to identify and characterize the yeasts. Isolates identified by biolog were belongs to genera *Saccharomyces, Candida humilis* and *Kluyveromyces* and found to utilize glucose, sucrose, raffinose, trehalose and maltose. Genetically, the sequence analysis of the ITS-5.8S rDNA region showed that the isolates belong to 5 species namely, *Saccharomyces cerevisiae, Candida humilis (synonyms Candida milleri), Kazachstania bulderi*, *Pichia fermentans* and *Pichia kudriavzevii.* All the test isolates were found to produce maximum cell mass at a temperature of 30^{o}C, pH of 5.5 and 48 hours of incubation. Isolate AAUTf1 did not produce hydrogen sulfide. The isolates AAUTf5, AAUTj15 and AAUSh17 including commercial yeast produced light level of hydrogen sulfide, while AAUMl20 and AAUWt21 produced heavy level of hydrogen sulfide. The assessment of leavening performance of the yeasts indicated that, isolates AAUTf1 (*Candida humilis*) and AAUTf5 (*Kazachstania bulderi*) had higher leavening activity with raising dough by 131 cm^3 and 128 cm^3 respectively each within 120 min. Similarly, isolates AAUSh17 (*Saccharomyces cerevisiae*) and AAUTj15 (*Saccharomyces cerevisiae)*, were found rising the volume of the dough by 127 cm^3 and 125 cm^3, respectively at 60 min compared to the commercial yeast that increased the volume to 117 cm^3 within 90 min. The combined bread dough rising effects of AAUTf1 + AAUTf5 + AAUTj15, AAUTf5 + AAUTj15 and AAUTf1 + AAUTj15 + AAUSh17 caused volume increment to 143 cm^3 at 90 min, 141 cm^3 at 60 min and 140 cm^3 at 60 min, in that order. Generally, mixed cultures showed superior leavening potential than that of single cultures and commercial yeast.

Keywords: Commercial yeast, Dough fermentation, Internal transcribed spacer, Leavening activity

1. INTRODUCTION

1.1. Background information

The global population is growing and is expected to reach 9 billion people by the middle of this century. One of the consequences of this growth in population is a higher consumption and a greater demand for processed food such as bread (Godfray *et al*., 2010). The increasing demand of bread as a staple food of human consumption has led to the development of the baker's yeast industry (Nasr and Zaky, 2011).

Baker's yeast (*Sacharomyces cerevisiae*) is the common name for the yeast commonly used as a leavening agent in baking bread and bakery products, where it converts the fermentable sugars present in the dough into carbon dioxide and ethanol (Hamelman, 2004). The commercial preparation of baker's yeast consists of dried cells of one or more strains of the fungus *Saccharomyces cerevisiae* (Klieger, 2004). The fermentative activity of baker's yeast is essential not only for the rising action of the dough by production of carbon dioxide, but also in production of the wide range of aroma compounds identified in bread (Birch *et al*., 2013).

According to Pattison and Von-Holy (2001), the production of carbon dioxide increases the dough size thus giving bread its characteristics of being light with a spongy texture. It is very indicated that the yeast is so vital in baking industry that its functions cannot be substituted with any other component or ingredient. Alcamo (2001) also reported that fermentation of bread involves the activities of alpha and beta amylases present in the moistened dough to release from the starch, maltose and sucrose.

The useful physiological properties of yeast allowed a wide variety of biotechnological applications, such as in many food and beverage industries, in bread making and in single cell protein production (Dai *et al*., 2014). In addition, yeast is a valuable source of protein and vitamins, especially certain aminoacids like lysine, methionine and threonine and B-group vitamins (Kamel and Stauffer 1993; Burrows 1979).

Jahan *et al*. (2007) have successfully assessed *Saccharomyces* species from different sources such as decomposed bread, fermented rice and fermented fruit juices and were considered as

potential bakers' yeasts on the basis of their ability to produce high amount of biomass and leavening action.

There are different sources for the isolation of yeast species (Qureshi *et al.*, 2007). Mostly found from fermented foods and beverages (Mogesse, 2006, Aslankoohi *et al.*, 2016), the citrus juice (Arias *et al*., 2002), dahi (Savova and Nikolova, 2002), sugarcane juice (Antonnini *et al*., 2004), molasses (Rose, 1976) and Honey (Marvin, 1931).

Dechassa (2010) isolated and selected ethanol tolerant yeasts from *tella, tej,* honey and *teff* dough for efficient ethanol production and classified under genus *Saccharomyces* based on morphological appearance and physiological testing of carbohydrates. Similarly Tamene and Dawit (2014) also reported that, yeasts species of *Saccharomyces cerevisiae* isolated from *tella* and *teff* dough and compared to commercial yeast for their baking purpose. The yeasts isolated from *teff* dough have superior leavening capacity than of yeasts from *tella* sample but lower performance capacity of the commercial yeast.

1.2. **Summary of statement of the problem**

Baked foods are an important part of the Ethiopian gastronomy (widely consumed) and as such plays an important role in the local economy. The bakery sector is constantly growing in Ethiopia due to an increasing demand for bread (particularly commercially prepared bread), constant growth in income, population and urbanization and to the shift from traditional consumption habit to fast food. Moreover, a number of alcohol and beverage industries (beer and wine) are present and these industries need tremendous amounts of yeast. As a result the use of commercial baker's yeast is increasing day to day.

The supply of commercial yeast is currently met upon importation due to no baker's yeast producing plant in the country (Tamene and Dawit, 2014). The country spends 293,010,632 ETB (14,650, 531.6 US $) in 2016 (CSA, 2016) for the importing baker's yeast. This necessitates need for alternative import substitution of baker's yeasts for the national development since the raw materials (molasses and wild yeasts) necessary to isolate industrial yeasts are locally available.

In Ethiopia there are several fermented foods and drinks such as *enjera*, *difo dabo*, *shamita*, *tella*, *tej*, *kocho*, etc. And one of the predominant fermenting microorganisms found in these foods and beverages are yeasts (Mogessie, 2006, Mooha, *et al*., 2015). Molasses is a waste from the sugar production industry also dominated by yeasts (Rose, 1976). Growth kinetics, pattern and diversity of microbial fermentation of Ethiopian foods and beverages and evaluation of yeast biomass production are well documented (Mogessie, 2006; Tamene and Dawit, 2014; Getachew, 2015).

However, the leavening capacity of wild yeasts isolated from these substrates *(teff* dough, wheat dough, *shamita, tej, tella*, honey and molasses) and their identification at biochemical and molecular levels that pave the way to their commercial scale production was not done.

Therefore, it is necessary to isolate and develop good performing baker's yeast, which would fulfill this demand. Therefore, the present work was aimed at isolating, identifying and characterizing yeast species from different locally available substrates. These resources include molasses, honey, *teff* and wheat fermented dough, *tej, tella* and *shamita*. Potential yeast isolates from these sources were isolated and selected to be used in bakery on the basis of their biomass yield, fermentation ability and better leavening action.

1.3. OBJECTIVES OF THE STUDY

1.3.1. General objective

The general objective of this study was to find potential indigenous baker's yeasts from local fermented foods, drinks and sugar byproducts that exhibit desirable characteristics of baker's yeasts.

1.3.2. Specific objectives:

The specific objectives of this study were to:

- Isolate, identify and characterize indigenous wild yeasts from fermented *teff* and wheat dough, *tej*, *tella*, *shamita*, molasses, and honey;
- Optimize the cultivation condition and their interaction effect on yeast growth and
- Evaluate dough fermenting abilities of the single and in combination of wild yeasts and compared to the commercial baker's yeast,

2. REVIEW OF LITERATURE

2.1. Fermented foods as sources of wild yeasts

Food fermentation is one of the oldest (Kalui *et al*., 2010; Agaliya and Jeevaratnam, 2013; Rhee, *et al.*, 2011) and most economical (Desiye and Abegaz, 2013; Erbas *et al*., 2006) methods of food processing and preservation (Kalui *et al*., 2010; Agaliya and Jeevaratnam, 2013; Altay *et al.*, 2013), as well as being a precursor of modern biotechnology (Kohajdova, 2010). Indigenous fermented foods such as bread, cheese and wine, have been prepared and consumed for thousands of years and are strongly linked to culture and tradition, especially in rural households and village communities (Mike and Sue, 1998).

Microorganisms have been playing a pivotal role in the fermentation of human foods and beverages since the beginning of human civilization. Fermented foods and beverages are defined as products obtained through desirable biochemical changes caused by the action of microorganisms (Aloys and Angeline, 2009) and their enzymes (Aloys and Angeline, 2009; Campbell-Platt, 1994). In indigenous fermented foods, the microorganisms responsible for the fermentation are usually the microflora naturally present on the raw substrate (Mogesse, 2006).

It has been indicated that fermentation adds safety and nutritional value to the food (Agaliya and Jeevaratnam, 2013; Navarrete-Bolanos, 2012) and improves their palatability (Nyanzi and Jooste, 2012). It also increases the shelf life (Egwim *et al*., 2013; Nout 2009) of foods and makes them more digestible (Agaliya and Jeevaratnam, 2013; Nout, 2009). All over the world, fermented foods and beverages continue to provide an important part of our diet (Campbell-Platt, 1994).

Yeast species are functional flora in indigenous fermented foods such as bread, cheese, wine, sourdoughs, fermented meats, and fermented vegetable products (Table1; Johnson and Echavarri-Erasun, 2011). Fermenting yeasts play an important role in the food industry as they produce enzymes that favor desirable chemical reactions such as the leavening of bread and the production of alcohol and invert sugar (Joseph and Bachhawat, 2014). The most beneficial yeasts in terms of desirable food fermentation are from the *Saccharomyces* family (Mike and Sue, 1998).

Table 1. Representative Yeast Genera and Species Associated with Fermented Foods and Feeds

Food	Yeast Species Involved	Food	Yeast Species Involved
Beers and ales	*Saccharomyces cerevisiae, Saccharomyces bayanus Saccharomyces pastorianus, (S. carlsbergensis) Saccharomyces bayanus var. uvarum*	Fermented olives and cucumbers	*Candida valida, Saccharomyces bayanus, Yarrowia lipolytica, Debaryomyces* spp.,*Candida* spp., *Kluyveromyces marxianus, Lachancea cidri, Pichia* spp., *Rhodotorula* spp., *Saccharomyces* spp.
Breads and bakery products	*Saccharomyces cerevisiae, Torulaspora delbrueckii Candida krusei (sourdough), Candida milleri (sourdough), Kazachstania exigua (sourdough), Zygosaccharomyces rouxii*	Kenkey (African fermented maize)	*Candida* spp., *Debaryomyces* spp., *Kluyveromyces* spp., *Saccharomyces* spp., *Trichosporon* spp.
Cachaca	*Saccharomyces cerevisiae, Schizosaccharomyces pombe*	Kimchi	*Candida spp., Cryptococcus* spp., *Saccharomycopsis* spp., *Kluyveromyces* spp. *Pichia* spp., *Rhodotorula* spp.
Cheeses	*Debaryomyces hansenii, Candida* spp., *Kluyveromyces marxianus, Kluyveromyces lactis, Yarrowia lipolytica, Geotrichum candidum,*	Probiotics	*"Saccharomyces boulardii", Saccharomyces cerevisiae, Kluyveromyces marxianus, Candida* spp.
Other dairy products (e.g., kefir; yoghurt, fermented milk)	*Kluvyveromyces marxianus, Candida kefyr, Candida famata, Candida krusei, Debaryomyces hansenii, Geotrichum candidum, Yarrowia lipolytica*	Soy paste (Chiang; Miso)	*Zygosaccharomyces* spp., *Candida* spp.
Ciders	*Saccharomyces cerevisiae, Saccharomyces bayanus*	Soy sauce (Jiang yu; Shoyu)	*Zygosaccharomyces rouxii,Candida famata,Candida etchellsii, Candida versatilis, Debaryomyces* spp. *Other Candida* spp.
Cocoa	*Saccharomyces cerevisiae, Hanseniaspora uvarum Kloeckera apiculata, Kluyveromyces marxianus, Pichia fermentans*	Tea fungus	*Zygosaccharomyces Kombuchaensis, Candida* spp.
Coffee	*Kluyveromyces marxianus, Saccharomyces bayanus, Schizosaccharomyces* spp., *Candida boidinii*	Wines	*Saccharomyces cerevisiae, Saccharomyces bayanus Saccharomyces bayanus var. uvarum, Saccharomyces kudriavezii, "Saccharomyces sake" Other Saccharomyces* spp., *Hanseniaspora uvarum*
Fermented meats and sausages	*Debaryomyces hansenii, Candida spp., Cryptococcus* spp., *Rhodotorula* spp., *Saccharomyces cerevisiae, Yarrowia lipolytica*	Silage	*Candida* spp., *Cryptococcus* spp. *Pichia spp., Saccharomyces spp., Trichosporon* spp.

Primary sources: Bekatorou *et al*., 2006; Walker, 2009; Johnson and Echavarri-Erasun, 2011.

2.2. General characteristics of baker's yeasts

2.2.1. Yeast: Their Biology and Ecology

Yeasts are eukaryotic microorganisms classified as members of the fungus kingdom with currently identified 1,500 species (Kurtzman and Fell, 2006) and additional 669,000 extant yeast species have not yet been described (Verstrepen *et al*., 2006). The most important yeast species for fermentation technology belong to the genus *Saccharomyces* and are taxonomically grouped in the *Saccharomyces sensu stricto* complex (Rainieri *et al*., 2003; Vaughan-Martini and Martini, 2011).

DNA sequencing has revolutionized yeast taxonomy. About 40 different yeast species have been sequenced so far and genomic-level aspects of yeast evolution are gradually being unveiled. Most attention has been focused on the *Saccharomycotina* or *Hemiascomycetes* (Casaregola *et al*., 2011).

Yeasts are unicellular, although some species may also develop multicellular characteristics by forming strings of connected budding cells known as pseudohyphae or false hyphae (Kurtzman and Fell, 2005). Yeast sizes vary greatly, depending on species and environment, typically measuring 3–4 µm in diameter, although some yeast can grow to 40 µm in size. Most yeasts reproduce asexually by mitosis, and many do so by the asymmetric division process known as budding (Walker *et al*., 2002).

Yeasts are widely dispersed in the environment and are often from sugar-rich materials. They are commonly found in water, soil, plant leaves and flowers, fruits, foods and air. And grow typically in moist environments where there is an abundant supply of simple, soluble nutrients such as sugars and amino acids (Walker, 2009). Lachance and Starmer, 1998 also reports that, they are found in widely different aquatic and terrestrial sources, the atmosphere as well as certain restricted habitats. They may also be found associated with the body of certain animals since they act as intestinal commensal (Kurtzman and Fell, 1998).

2.2.2. Yeast Nutrition and their Metabolism

Yeast cells require macronutrients (sources of carbon, nitrogen, oxygen, sulfur, phosphorus, potassium, and magnesium) at the millimolar level in growth media, and they require trace elements (Ca, Cu, Fe, Mn, and Zn), at the micromolar level. Most yeast grows quite well in simple nutritional media, which supply carbon–nitrogen backbone compounds together with inorganic ions and a few growth factors (Walker, 2009).

Yeast cells are facultative anaerobes meaning that they can respire and survive under both aerobic and anaerobic conditions, there by not necessarily requiring oxygen for their both growth. In the absence of oxygen, they can metabolize sugar into alcohol (ethanol); carbon dioxide and low biomass (Figure 1). In well aerated conditions, the cells could be able to get enough energy and convert sugar into carbon dioxide, water and biomass (Figure 1) (Rose and Harrison, 1969; Bekatorou *et al*., 2006).

Therefore, yeasts can grow and yield higher biomass under aerobic conditions. In anaerobic conditions, as in alcoholic fermentation, yeasts do not grow efficiently, and sugars are converted to intermediate by-products such as ethanol, glycerol and CO_2. Therefore, in yeast propagation, the supply of air is necessary for optimum biomass production. The main carbon and energy source for most yeast is glucose supplied from molasses, which is converted to the glycolytic pathway to pyruvate and by the Krebs cycle to anabolites and energy in the form of ATP (Balls *et al.,* 2007).

Yeasts can metabolize various carbon substrates but mainly utilize sugars such as glucose, sucrose and maltose. Sucrose is metabolized after hydrolysis into glucose and fructose by the extra cellular enzyme invertase. Maltose is transferred in the cell by maltose permease, and metabolized after hydrolysis into two molecules of glucose by maltase. Some yeast can utilize a number of unconventional carbon sources, such as biopolymers, pentoses, alcohols, hydrocarbons, fatty acids and organic acids (Bailey *et al.,* 1977).

Although yeasts cannot fix molecular nitrogen, simple inorganic nitrogen sources such as ammonium salts are widely used. Ammonium sulfate is commonly used nutrient in yeast

growth media because it provides a source of both assimilable nitrogen and sulfur. A variety of organic nitrogen compounds (amino acids, peptides, purines, pyrimidines, and amines) can also provide the nitrogenous requirements of the yeast cell. Glutamine and aspartic acids are readily deaminated by yeasts and therefore act as good nitrogen sources (Walker, 2009).

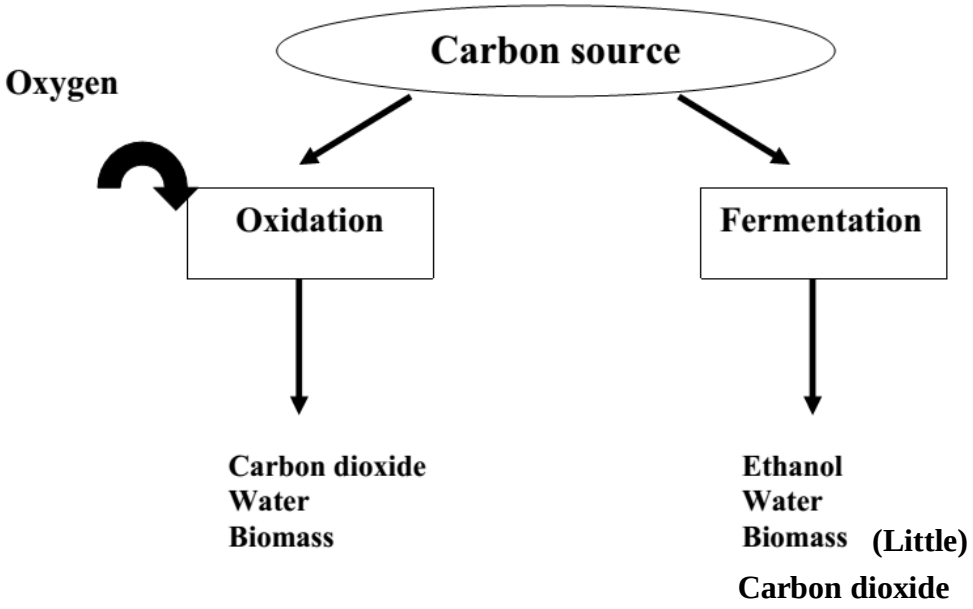


Figure 1. Yeast metabolism (Modified from Rose and Harrison, 1969; Bekatorou *et al.*, 2006).

2.2.3. Yeast reproduction and cell cycle

Yeast typically grow asexually by vegetative multiplication but can also reproduce sexually by forming ascospores (Figure 2). The cell cycle in budding or vegetative multiplication consists of four distinct phases (G1, S, G2 and M). The sexual reproduction involves the formation of four haploid spores (two MATa and two MATα) and is induced during nutrient starvation (Taxis *et al*., 2005). During conjugation, two cells of opposite mating type (MATa and MATα) fuse to form a diploid zygote (Jackson and Hartwell, 1990). Strains that can be maintained stably for many generations as haploid are termed heterothallic. Strains in which sex reversals, cell fusion and diploid formation occur are termed homothallic. The large majority of *S. cerevisiae* industrial strains are homothallic (Clara, 2015).

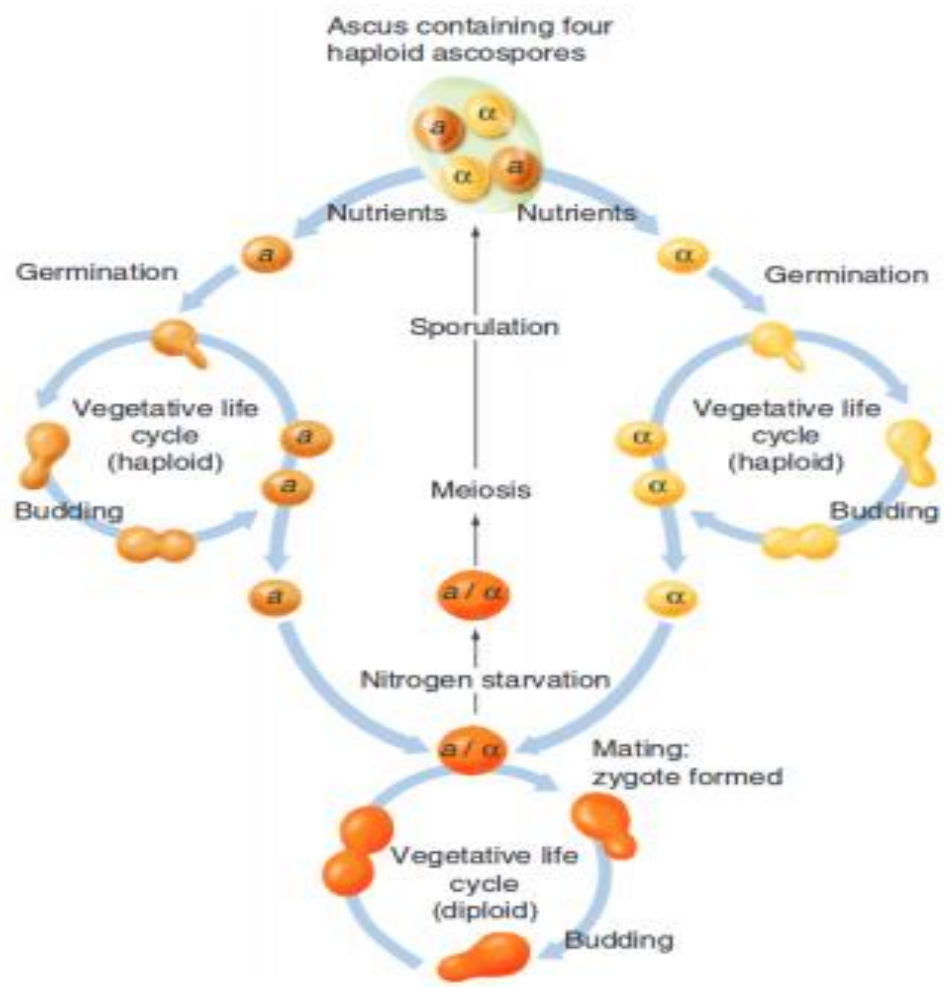


Figure 2. Life cycle of yeasts. (Clara, 2015).

Yeast population growth is the result of cell division and the progression through the cell cycle. Under optimal growth conditions, yeast growth kinetic follows the typical microbial growth curve, comprising four main phases: lag phase, exponential phase, stationary phase and death (decline) phase (Figure 3; Clara, 2015). The **lag phase** reflects the time required for yeast cells to adapt to their new environment by synthesizing ribosomes and enzymes needed to establish growth at a higher rate. The duration of this phase depends on firstly the initial population size and secondly environmental conditions. Once the cell starts actively metabolizing, they begin DNA replication and shortly after the cells divide. This begins the second phase of growth called the **exponential phase (Log phase)** of growth. This is the period in which the cells reproduce at maximum specific growth rate (μmax). The time it takes the population to double is called generation time. Yeast strain, growth medium, and temperature are

important factors in determining the generation time. Industrial fermentations aim to extend this phase for maximizing the output of biomass and metabolites production (López *et al*., 2004). The third phase in yeast growth is the **stationary phase**; a period of no growth when metabolism slows and cell division is stopped. The factors that cause cells to enter stationary phase are related to change in the environment, such as nutrient deprivation, toxic metabolites and high temperatures. After prolonged periods in stationary phase, cells may die and autolysate (Clara, 2015).

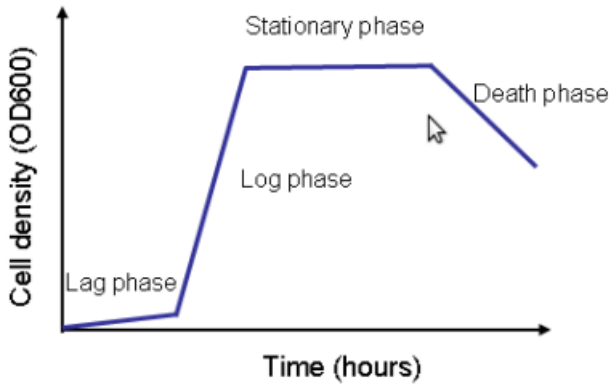


Figure 3. Standard yeast growth curve (Clara, 2015)

2.3. Ethiopian indigenous fermented foods and beverages and their dominant microorganisms

Fermented foods are food substrates that are invaded or overgrown by edible microorganisms whose enzymes, particularly amylases, proteases, and lipases, hydrolyze the polysaccharides, proteins, and lipids to nontoxic products with flavors, aromas, and textures that are pleasant and attractive to the human consumer (Steinkraus, 2009). All over the world, fermented foods and beverages continue to provide an important part of diet (Campbell-Platt, 1994). Their consumption has increased greatly since the 1970s, because they are considered as healthy and natural products (Navarrete-Bolanos, 2012).

Fermented food products form significant part of the diet of many indigenous communities in the developing world (Parkouda *et al*., 2009). Fermented foods and beverages are a good source of yeasts and lactic acid bacteria. African indigenous fermented food products, like many fermented food products in different parts of the world are deemed to have improved flavour, texture,

increased shelf-life, bioavailability of micronutrients, and reduced or absence of anti-nutrition and toxic compounds among others (Sefa-Dedeh *et al*., 2004).

Ethiopia is one of the countries where varieties of traditional fermented foods and beverages are produced and consumed. The major indigenous fermented foods which are produced in Ethiopia are *injera, difo dabbo, shamita, tella, tej* etc. (Mogesse, 2006). Injera is a fermented sour leavened staple food consumed widely in Ethiopia (Umeta *et al*., 2005; Samuel, 2015). It is a soft porous thin pancake prepared from tef (*Eragrostis tef*), an ancient cereal, indigenous to Ethiopia (Yigzaw *et al*., 2001; Schneider and Anderson, 2010). Sour dough bread (locally known as *Difo dabbo*) occupies a prominent place in the Ethiopian diet and continues to play an important socio-economic role. The main raw materials employed are wheat supplemented with tef (*Eragrostis tef*) flour or barley flour and their primary micro flora are yeasts, a simultaneous souring action takes place due to the activities of the lactic acid bacteria present (Kelbessa Urga and Narasimha, 1998). *Shamita* is a very popular traditional fermented drink in Ethiopia with low alcohol content and mainly consumed as meal replacement by most people who cannot afford a reasonable meal. This beverage made up of barley flour, salt, ground linseed and spices mixed with water together. The fermenting organisms were composed of yeasts and lactic acid bacteria (Mogesse, and Mehari, 1995; Mogesse, 2006).

One of the most consumed fermented alcoholic beverages is *tella* (traditional beer), which is made mostly with barley but wheat, maize, sorghum, and *teff* are utilized depending on the region. The main fermenting yeast is *Saccharomyces cerevisiae* and saccharification of cereal starch seems to depend on malt (Lee *et al*., 2015). *Tej (*honey wine) is an indigenous drink which is prepared from honey, water, and leaves of *gesho (Rhamnus prinoides*) (Bekele *et al*., 2001). Yeasts of the genus *Saccharomyces* were reported to be responsible generally for the conversion of sugars to ethanol in *tej* (Vogel and Abeba, 1983).

2.4. Yeasts and fermentation

2.4.1. Importance of yeasts in fermentation

Yeasts have benefitted humankind for millennia in scientific, food, medical, and agricultural disciplines (Figure 4; Johnson and Echavarri-Erasun, 2011). Yeasts are part of

the microflora of many indigenous foods. In most cases, the microflora consists of yeasts and bacteria, and in some cases molds. In addition to the fermentation of the foods, the combination of these organisms adds to the distinctive texture, aroma, and flavor (Joseph and Bachhawat, 2014).

Saccharomyces genus possesses unique characteristics in fermenting sugars vigorously, either in the presence or in the absence of oxygen, to produce ethanol. This ability allows them to colonize sugar-rich substrates (plant saps and fruits) and compete with other yeasts, which are not so tolerant to alcohol (Vaughan-Martini and Martini, 1998). The apparition of angiosperm plants with sugar-rich saps and fruits introduced a new ecological niche with a different selection regime that likely imposed altered physiological demands to the ancestors of *Saccharomyces* yeasts (Wolfe and Shields, 1997).

Baker's yeast, used for bread fermentation throughout the world, is very important for the bread quality and different commercial bakery products. The fermentative activity of baker's yeast is essential not only for the rising action of the dough by production of carbon dioxide, but also in production of the wide range of aroma compounds identified in bread (Birch *et al*., 2013; Frasse *et al.*, 1992; Schieberle and Grosch, 1991).

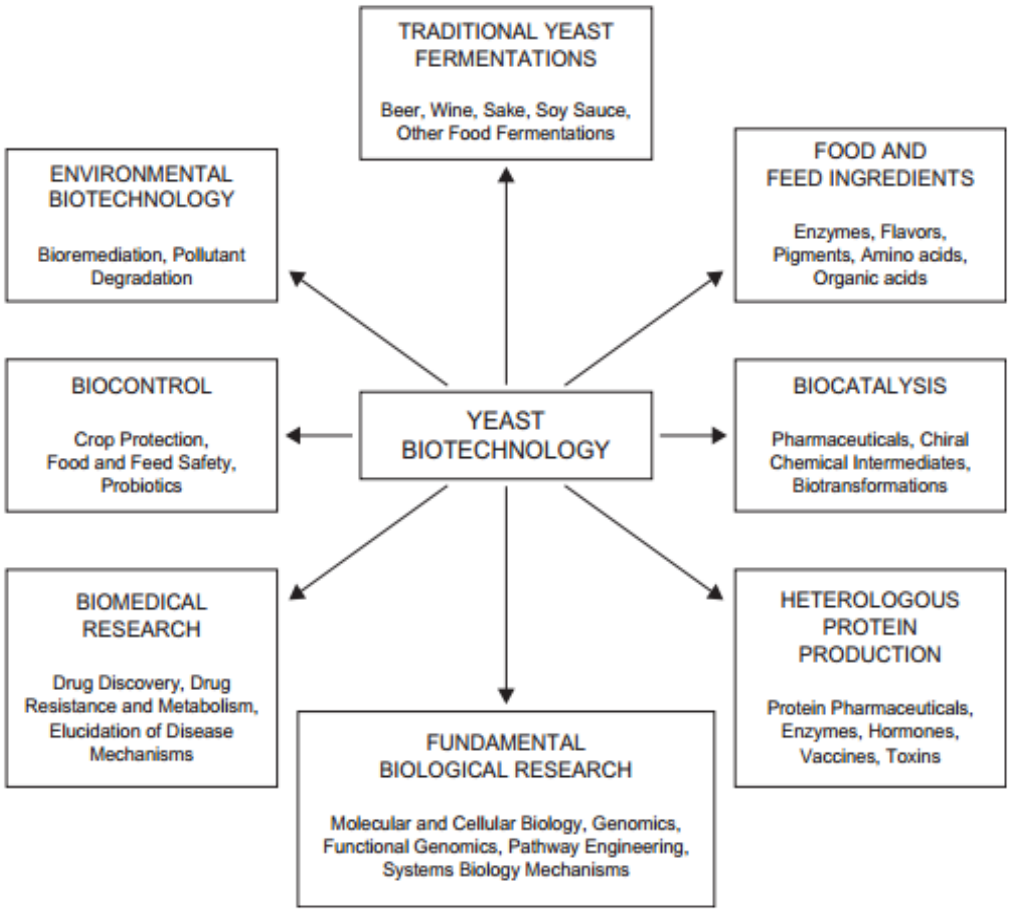


Figure 4. Disciplines in yeast biotechnology (Johnson and Echavarri – Erasun, 2011).

2.4.2. Yeasts and bread fermentation

Bread is a major nutritional component and bread making is one of the oldest processes, known and practiced for thousands of years, worldwide (Plessas *et al.*, 2005). The primary microorganisms involved in bread making are yeasts and their role are leavening of dough. Leavening is the increase in the size of the dough induced by the metabolism of yeasts. The primary role of the baker's yeast lies in the leavening of the dough as a result of the buildup of CO_2, a product of fermentation of sugars (Donalies *et al.*, 2008; Edwards, 2007).

During bread making yeasts ferment hexose sugar mainly in to alcohol, carbon dioxide and smaller amounts of glycerol. The carbon dioxide dissolves continuously in the dough, until the latter becomes saturated. Subsequently, the excess carbon dioxide in the gaseous state begins to form bubbles in the dough. It is this formation of bubbles which causes the dough to rise or to leaven. The Total time taken for the yeast to act upon the dough varies from 2-6 hours (Okafor,

2016). *Saccharomyces cervisiae* is the most commonly used species of *Saccharomyces* in bread baking and it has been employed as baker's yeast in manufacturing bread for at least 6,000 years (Alcamo, 2001; Bell and Attifield, 2001).

Saccharomyces strains are highly domesticated and being used in industry but much of the potential of the natural diversity of yeasts remains unexplored (Steensels *et al*., 2014; Gallone *et al*., 2016). Currently, new research attempts that other yeast species such as *C. humilis* (synonym *C. milleri)* (Haggman and Salovaara, 2008), *Kazachstania bulderi* (Lhomme *et al*., 2016), *Kazachstania gamospora* and *Wickerhamomyces subpelliculosus* (Zhou *et al*., 2017), *Torulaspora delbrueckii* and *Saccharomyces bayanus* (Almeida and Pais, 1996; Aslankoohi *et al*., 2016), *Schizosaccharomyces pombe*, *Dekkera bruxellensis* (the hybrid *Saccharomyces carlsbergensis*) and other *Saccharomyces* (Hagman *et al*., 2013; Merico *et al*., 2007; Xiao *et al*., 2015) species have been reported as candidate of leavening agents. These yeasts have a huge potential for the discovery of novel alternative yeasts with dough leavening abilities.

2.4.3. Factors affecting fermentation of dough using yeasts

Bread dough fermentation is one of the key unit operations in bread making. Yeast metabolizes flour sugars into carbon dioxide gas which diffuses toward the air nuclei embedded in the dough during mixing (Scanlon and Zghal, 2001). There are several factors that influences dough fermentation using yeasts but the major are the yeast strain used, resistance to osmotic pressure, maltose adaptation, yeast concentration, flour fermentative capacity, temperature, pH of the dough presence of dough inhibitors and aeration (Collado-Fernandez, 2003).

Strain type: The selection of yeast strain is a function of fermentative properties of the dough, the bread making process, the high stability of its characteristics and its good industrial yield (Collado-Fernandez, 2003).

Osmotic Pressure: Some yeast strains have a high tolerance to osmotic pressure, but others do not and consequently die. The increase in osmotic pressure in the cell wall is due to factors such as salt and sugar concentration, the invertase action of the yeast itself. The decrease in water in the formulation of the doughs that include high levels of fat and sugar, and especially problems

presented by frozen dough sugar; during freezing, the free water decreases, and the osmotic pressure increases and affects yeast growth (Collado-Fernandez, 2003).

Maltose adaptation: In order to maintain a good production of gas, yeast requires a plentiful and continuous supply of glucose as an energy source. The action of alpha and beta amylases of flour in damaged starch gives maltose (starch damage results from modifications in the structure of starch during the milling process) (Randez-Gil *et al*., 1999; Collado-Fernandez, 2003). This disaccharide is transported into the cell, in which it is broken down into two molecules of glucose. The metabolic changes to this new source of glucose from starch are only active when yeast detects the presence of maltose in the medium, and its functioning takes some time (lag phase). Consequently, the rate of gas production decreases until this mechanism is working fully, and then, gas production increases (Collado-Fernandez, 2003).

Yeast concentration: When sugar is not added to dough, the limiting factor of gas production is the quantity of maltose available. The increase in yeast concentration at one temperature leads to an increase in the rate of gas production and greater sugar depletion (Collado-Fernandez, 2003).

Fermentative capacity of flour: The quantity of amylase in flour determines the degree of glucose production from starch, so long as there is enough damaged starch (Collado-Fernandez, 2003).

Temperature: the metabolic activities of yeasts are greatly affected by the temperature at which they grow; however, temperatures above the optimum cause's lower growth rate reduce oxygen solubility and change in the cellular composition of yeasts. On the other hand, rising of temperature (38^0C) increases gas production, and also results lactic and butyric fermentation (which are undesirable) (Collado-Fernandez, 2003). Choi *et al*., (2010); Nitayavardhana *et al*., (2010) reported that yeasts can grow at elevated temperatures of 40^0C, but the optimal temperature is approximately 30^0C.

pH: most yeasts grow very well between pH 4.5 and 6.5 (Walker, 2009). Usually, the pH of dough is 5, although the pH may be lower when sourdough is used. The undissociated forms of organic acids produced from parallel fermentation to alcoholic fermentation, that is, produced by lactic acid bacteria of flour and by contamination of yeast, are inhibitors of fermentative activity. They have greater effects at lower pH, the critical zone being between 4 and 5, and these values correspond to the final fermentation (Collado-Fernandez, 2003).

Presence of Dough Inhibitors**:** The copper and chlorine present in water used in bread making processes can act as fermentation inhibitors. Organic acids, such as those used as preservatives (acetic and propionic acid) and others formed during fermentation, can also act as fermentation inhibitors (Collado-Fernandez, 2003).

Aeration**:** Yeasts are facultative anaerobes, and can grow with or without oxygen. In the presence of oxygen, they convert sugars to CO_2, energy and biomass. In anaerobic conditions, as in alcoholic fermentation, yeasts do not grow efficiently, and sugars are converted to intermediate byproducts such as ethanol, glycerol and CO_2. Therefore, in yeast propagation, the supply of air is necessary for optimum biomass production (Bekatorou *et al.,* 2006).

2.4.4. Forms of baker yeast available for use

Commercial production of yeast is based on growth on an industrial scale of a pure selected strain. Baker's yeast is commonly available in six different forms, cream, compressed, active, instant, rapid rise and deactivated yeasts. These variations are related to the yeast moisture content (Young and Stanley, 2007*)*.

- **Cream yeast** is a form of commercial yeast preparation in which centrifugation and washing steps of the fermentor/yeast broth, into a yeast cream with a solids concentration of approximately 20 %. The advantage of cream yeast is that it excludes any human handling thus reducing the risk of contamination by handling. However, due to its high (water) volume, transport costs can be expensive (Akbar *et al*., 2012).
- **Compressed yeast** is cream yeast with most of the liquid removed. It is a soft solid, beige in color, and best known in the consumer form as small, foil-wrapped cubes of cake yeast. It is also available in larger-block form for bulk usage (Gisslen, 2008).
- **Active dry yeast** is granules yeast, with live yeast cells encapsulated in a thick jacket of dry, dead cells with some growth medium. Under most conditions, active dry yeast must first be rehydrated. It can be stored at room temperature for a year, or frozen for more than a decade, which means that it has better keeping qualities than other forms (Reinhart, 2001).
- **Instant yeast** is similar to active dry yeast, but has smaller granules with substantially higher percentages of live cells per comparable unit volumes. It is more perishable than

active dry yeast but also does not require rehydration. The main difference between active dry yeast and instant dry yeast is that active dry yeast has to be dissolved in warm water before usage, but instant does not for providing the activation (Bekatorou *et al.*, 2006).

- **Rapid-rise yeast** is a variety of dried yeast (usually a form of instant yeast) that is of a smaller granular size, thus it dissolves faster in dough, and it provides greater carbon dioxide output to allow faster rising (Kay,1997).
- **Deactivated yeast** is a product without leavening properties, used for the conditioning of dough properties in baking or the development of characteristic flavor (Bekatorou *et al.*, 2006). Inactive dry yeast typically used for pizza and pan bread dough, it is used at a rate of 0.1% of the flour weight, though manufacturer specifications may vary. (San Francisco Baking Institute Newsletter, 2003).

2.5. Yeast Isolation and Identification

2.5.1. Yeast Isolation

It is quite easy to grow yeasts in the laboratory on a variety of complex and synthetic media. Malt extract or yeast extract supplemented with peptone and glucose (as in YEPG) is commonly employed for the maintenance and growth of most yeast. Yeast nitrogen base (YNB) is a commercially, available chemically defined medium that contains ammonium sulfate and asparagine as nitrogen sources, together with mineral salts, vitamins, and trace elements. For the continuous cultivation of yeasts in chemostats, media that ensure that all the nutrients for growth are present in excess except one (the growth-limiting nutrient) are usually designed (Walker, 2009).

Yeasts usually occur in the presence of molds and/or bacteria (Yarrow, 1998), necessitating use of selective techniques for their recovery. In yeast isolation, the media used should be selective (permit the yeast to grow) while suppressing molds and bacteria. *Saccharomyces* can be isolated from natural sources and maintained in pure culture by conventional microbiological techniques. The source material (fermenting sugary materials, fruit juices or soil) usually is diluted serially and plated onto potato dextrose agar (PDA) or yeast extract–peptone–dextrose agar (YPDA). The growth of yeasts in preference to bacteria is achieved by the pH of the medium being below

neutral (usually 4–6) and the incorporation of antibacterial antibiotics (Joseph and Bachhawat, 2014).

Most isolation methods require the use of media, which is acidified using either hydrochloric acid or phosphoric acid like yeast malt agar acidified to a pH of 3.7. Acidified media are preferred over media incorporated with 20 antibiotics and fungistatic agents; fungistatic agents should be used with caution since it may also inhibit certain yeasts (Lachance and Starmer, 1998).

2.5.2. Yeast Identification

Classical culture-based diagnostic methods use morphological (size, color and shape of the colony), as well as biochemical (fermentation of selected carbohydrates, assimilation of carbon or nitrogen from selected organic compounds, acid production, etc.) characteristics of yeasts. These methods, however, require long time waiting for the final score, some lasting up to 1–2 weeks. Therefore, laboratories are increasingly choosing rapid diagnostic tests, such as OmniLog microplate, API® Candida, API® 20C AUX, and ID32C® that shorten identification time to 24–48 h. Commercial tests are based on evaluation of selected biochemical properties with assigned values, which in turn are given a numerical code designating the species (Katarzyna, 2011).

The major limitation of rapid diagnostic methods is that they are designed mainly for the identification of medical yeast isolates and their application is generally restricted to few yeast species (Arias *et al*., 2002). However, yeast identification using molecular techniques based on 5.8S-ITS restriction analysis and sequencing has proven to be a rapid, reliable, and accurate tool for environmental yeast identification (Fernandez-Espinar, 2000; Arias *et al*., 2002; Kurtman and Robnett, 1998).

Internal transcribed spacer (ITS) region is composed of two noncoding regions ITS1 and ITS2, which are separated by the highly conserved 5.8S rRNA gene (White *et al*., 1990) and is located between 18S and 28S rRNA genes (Figure 5). Esteve-Zarzoso *et al.,* (1999), established a database containing the 5.8S-ITS region endonuclease restriction patterns of 132 yeast species isolated from numerous sources. This 5.8S-ITS database combines reference yeast strains from

different origins (foods and beverages) and can be more useful for environmental or wild yeast strain identification than the clinically oriented commercial databases (Arias *et al.*, 2002).

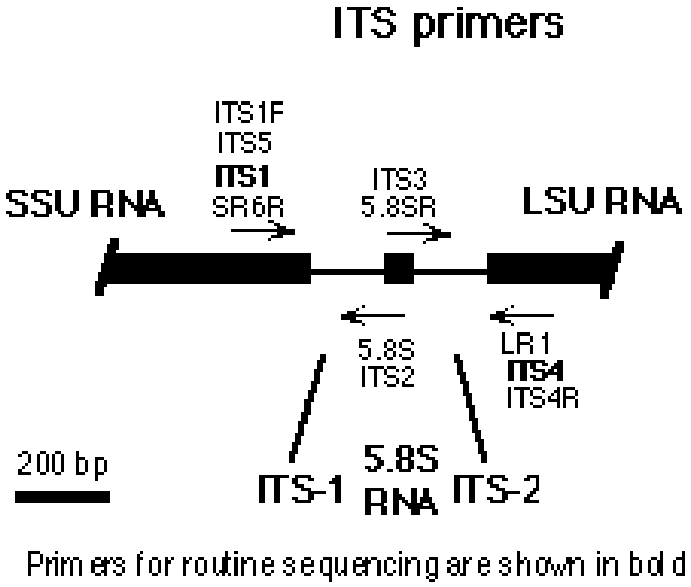


Figure 5. Schematic representation of the internal transcribed spacer (ITS) region of ribosomal RNA (rRNA) (Adapted from White *et al.* 1990).

Sequencing of the internal transcribed spacers (ITS) occurring between the 18S and 28S rDNA genes was investigated in order to confirm the identification of yeasts at species level (Montrocher *et al.*, 1998; Oda *et al.*, 1997).The discriminative potential of the ITS1-5.8S-ITS2 DNA region to investigate yeast species boundaries was successfully used by some authors using RFLP analysis and sequencing (Egli and Henick-Kling, 2001; Las Heras-Vazquez *et al.*, 2003; Naumova *et al.*, 2003 and Fernandez-Espina *et al.*, 2000).

2.6. Important traits for baking applications

The quality requirements of baker's yeast are many. In addition to excellent and uniform dough leavening ability, a typical, good baker's yeast should be able to tolerate a fairly wide range of temperatures, and sometimes also varying pH and the presence of sugar, fat and preservatives, as well as being a good aroma producer, etc. (Van hoek *et al.*, 1998). In view of the primary role of baker yeast in dough, fermentative capacity (i.e. the specific rate of carbon dioxide production by yeast upon its introduction in dough) is a particularly important parameter (Benitez *et al.*, 1996).

There are a range of qualities that must be exhibited in order for any yeast strain to be economically useful. In industrial terms qualities needed include: efficient yielding good quality biomass, efficient dough leavening activities and production of good bread flavor characteristics in various bread-making conditions, robustness to with stand stresses encountered during production, transport and application, and having good keeping quality or shelf life, which is the ability of yeast biomass to maintain its dough-leavening activity in storage for long period of time (Attfield and Bell, 2003).

2.7. Baker's yeast improvement

Yeasts have been used for thousands of years to make alcoholic beverages, such as beer, wine, and sake, as well as for bread fermentation (steensels *et al.* 2014). More recently, *S. cerevisiae* has also been used in the bio ethanol industry and for the production of heterologous compounds, such as human insulin, hepatitis vaccines, and human papillomavirus vaccines (Hou *et al*. 2012). Nowadays the production of baker's yeast biomass represents a highly competitive multi-billion dollar global industry (Attfield and Bell, 2003).

Despite the intensive use of yeasts (*S. cerevisiae)* in biotechnological applications and industrial fermentations, there is still significant room for improvement: industrial processes are rarely using the most suited or best-performing strain. This is because many industrial strains are currently used because of historical grounds, rather than being carefully selected for a specific application, and are therefore often suboptimal for their purposes. Additionally, demands for increased productivity, wider substrate range utilization, and production of non-conventional compounds in industry, as well as changing consumer preferences, lead to a great interest in further improving the currently used industrial strains and the selection or development of strains with novel properties (Steensels *et al.* 2014).

The emerging knowledge of genetic engineering modifies the properties of baker’s yeast strains for improved performance and bread quality improvement (Osinga, 1992). Genetic modification of baker's yeast can be achieved by classical or molecular procedures, or a combination of both approaches. Although, classical strategies remain the most practical approach to develop strains for commercial applications, genomics and molecular techniques remain important for

determining key genes, pathways and associated physiological functions that need to be enhanced in novel strains of baker's yeast industry (Attfield and Bell, 2003; Steensels *et al.* 2014).

Self-cloning is defined as genetic modification where no DNA from another species is introduced into the genome. Therefore, the use of self-cloning principles may help to obtain formal approval to use the strain for production purposes (Steensels *et al.* 2014). The first genetically modified baker's yeast, developed at Gist Brocades (Delft, The Netherlands), was given approval by the UK's Advisory Committee on Novel Foods and Processes for mass production and use in bakeries (Lloyd-Evans, 1994). Nakagawa and Ouchii (1994) have also announced the development of a baker's yeast that has improved freeze tolerance and high fermentative activity in both lean and sweet dough's. Park *et al.* (2003) could significantly improve thermo tolerance, osmotolerance, or ketoconazole resistance yeasts by screening the mutants expressing artificial transcription factors.

2.8. Baker yeast demand in Ethiopia

Commercial yeast production worldwide exceeds 1.8 million tons per year (Joseph and Bachhawat, 2014). Depending on cultural habits, different types and forms of bread are consumed in large quantities throughout the world. It is estimated that over 1.8 billion people consume various kinds of flat breads globally (Chavan and Chavan, 2011). Yeasts are used mostly by the baking industry, but also by the brewing and distilling industries. Yeast is also a commercial source of natural flavorings and the dietary supplements (Bekatorou *et al.*, 2006).

Ethiopia is a developing country with a high of baker's yeast demand from a number of bakeries. The urban people are somewhat changing their food habit due to the fast food culture of the western world. One of the basic ingredients of these fast foods is bread. As a result the use of commercial baker's yeast is increasing day to day. Moreover, a number of alcohol and beverage industries (beer and wine) are built and these industries need tremendous amount of yeast (Tamene and Dawit, 2014) but there is no baker's yeast producing plant in the country.

Table 2 shows total amount of yeast (active and inactive) imported to Ethiopia, the cost and partner countries (CSA, 2016). As reported by the Central Statistics Authority (CSA, 2016) the country has imported a total of 4,841,271.9 kilograms active and inactive dry yeasts in the year 2015 and 4,919,908.9 kilograms of active and inactive dry yeast in the year 2016. The amount of yeast imported to the country in the year 2016 has increased by 78,637 kilograms from the 2015 import.

A considerable amount of foreign currency is spent to meet the annual demand of our bakery ingredients and alcohol industries. This is because baker's yeast is purchased with dollars and there is extra cost for transportation. For instance the national expenditure of money in Ethiopia upon importing active and inactive dry yeasts together in the years 2015 and 2016 as shown in Table 2 was 213,227,807 ETB (US $ 10,661,390.4) and 293,010,632 ETB (US $ 14,650,531.6) respectively. The country in 2016 has spent cost 79,782,825 ETB (US $ 3,989,141.25) from the 2015. Baker's yeast producing plant is needed to be established in our country to fulfill the demand and to save foreign currency.

Table 2. Amount of yeast (active dry and inactive dry) in kilograms, year imported, partner countries and the cost in ETB (CSA, 2016).

Year	2015				2016			
Type of yeast	Active dry yeasts		Inactive dry yeasts		Active dry yeasts		Inactive dry yeasts	
Source	Net wt in Kg	Birr	Net wt in Kg	Birr	Net wt in Kg	Birr	Net wt in Kg	Birr
United Arab Emirates	487	258818.8			434.2	45,180.7		
Belgium	301	582888.6			132	271,301.8		
Switzerland	126	20846.2						
China	1201404	56696236	377755.09	1,862,962	738,175.3	36,194,312	38,990.3	1,752,799.3
Germany			1.56	1,274.67	208	317,117		
Denmark	847.6	372899	40	125,216.2	2,578.2	568,191.3		
Egypt	1857373	90750906	644320	31,599,831	2,469,167	117,807,490	912,320	44,501,713
Estonia	307326.2	2182						
Spain							1715.6	484,049.1
France	2856	1374953	847.65	2,141,127	98,565	55,684,471		
United Kingdom	1097	198083.5	3	4,102.87	1	12,302		
Greek	7.7	3772.8						
Israel	1	239.01						
India	265	29120.12	1250	293,365.6	805	41,476.4		
Italy	554.69	12544.71			4563	589,943.8		
Malta	4520	246003			4415	187,134.1		
Netherlands	17.87	127569.5	12	159,295.4	72	773,501.4		
Sweden	55	210438						
Tunisia	34782	1801560						
Turkey	405324.5	24349635			647,746.8	33,739,501		
United States	2	1936.5						
South Africa					20	40,148.4		
Total	3,817,342.6	177,040,632	1,023,929.3	36,187,174.7	3,966,883	246,272,071	953,025.9	46,738,561.4

3. MATERIALS AND METHODS

3.1. Samples and Sampling

A total of seventy samples (Table 3) from seven substrates (fermenting *teff* and wheat dough, *shamita*, *tella*, *tej*, honey and molasses) were collected. To obtain representative isolates of the yeasts involved in the fermentation, ten samples from each type of fermenting *teff* dough at (48 and 72 hours) and wheat dough were sampled from twenty local houses in Addis Ababa. A total of thirty samples of *shamita, tella* and *tej* were collected randomly from vending houses in Addis Ababa, while ten samples of honey were collected from Addis Ababa, Jimma, Gondor, Guragueand Wukro. Ten samples of molasses were collected from Arjo-Dedesa, Fincha, Metehara and Wonji Sugar Factories (Table 3, Figure 6)**.** All the samples were collected in sterile screw-capped bottles, brought to the laboratory and then stored at 4^{o}C for further study.

The laboratory work was carried out at Addis Ababa University, College of Natural Science (Mycology laboratory) and at Addis Ababa Institute Technology (AAiT), Ethiopian Biodiversity Institute (EBI) and at Molecular Research Center (MRC-Eth) advanced laboratory in Addis Ababa, Ethiopia and United States of America.

Table 3. Summary of samples collected from various sites

Sample type	Sample Quantity	Sample area
Teff dough (*Eragrostis tef*)	10	Addis Ababa
Wheat dough	10	Addis Ababa
Shamita	10	Addis Ababa
Tella	10	Addis Ababa
Tej (Honey wine)	10	Addis Ababa
Molasses	10	Arjo-Dedesa, Fincha and Metehara Sugar Factories
Honey	10	Jimma, Gondor, Gurague,Wukro and Addis Ababa
Total	70	

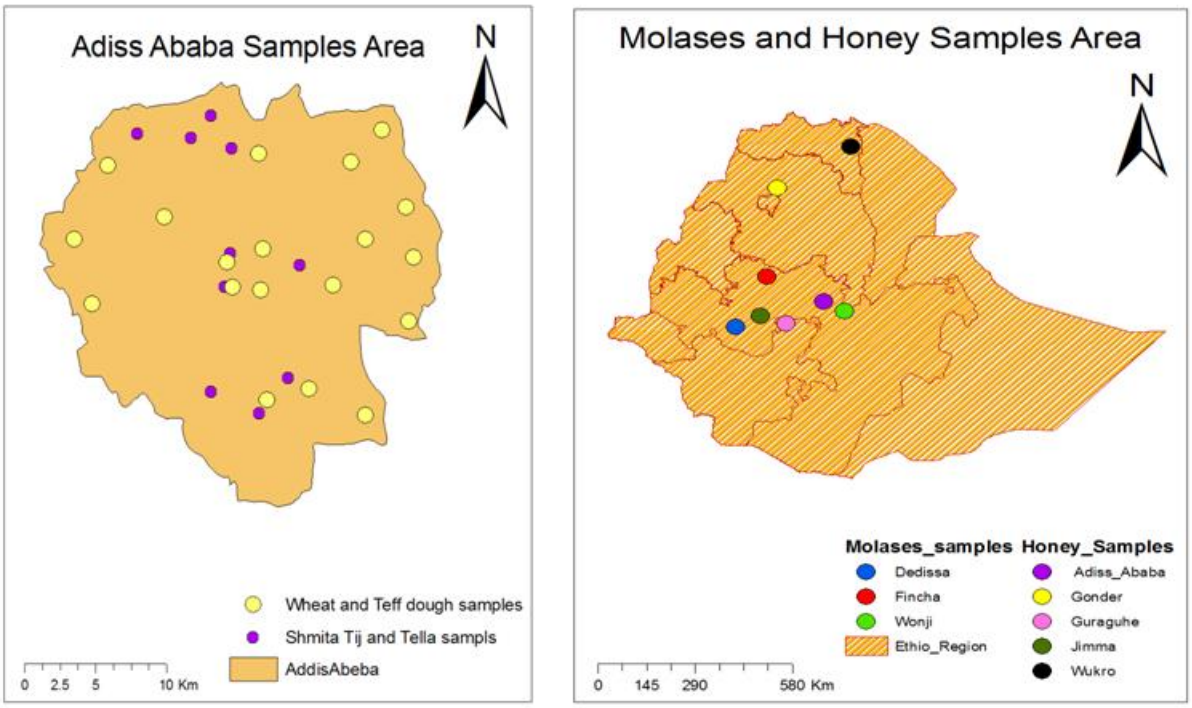


Figure 6. Map of sampling sites

3.2. Isolation, purification and screening of yeasts

3.2.1. Isolation and purification of yeasts

The isolation procedures for the seven substrates (Table 3) and active dry baker's yeast were done by serial dilution agar plate. Ten gram of each sample was mixed with 90ml of sterile distilled water in a sterile flask for serial dilutions. Then the mixture was homogenized for 30 min using shaker (120rpm) and one ml of the mix was taken for serial dilution and added into test tube containing 9ml sterile distilled water. This was followed by spread plating aliquots of 0.1ml from appropriate dilutions (10^{-1}-10^{-6}) on Rose Bengal Chloramphenicol Agar (15 g L^{-1} agar, 10 g L^{-1} glucose, 5 g L^{-1} papain-digested soybean meal, 1 g L^{-1} KH_2PO_4, 0.5 g $L^{-1}$$MgSO_4$ $7H_2O$, 0.05 g L^{-1} Rose Bengal, and 0.1 g L^{-1} chloramphenicol) plates in duplicate which is a selective medium for yeasts (Díaz *et al*., 2013). All the plates were incubated at 30^{o}C for 48 hours (Qureshi *et al*., 2007).

After incubation different colonies (three to five) were picked (each colony represented one isolate) on the basis of colony morphology (Barnett *et al*., 2000). The colonies picked were further purified by streaking on YEPDA (yeast extract, 10 g; peptone, 20 g; glucose, 20 g; Agar,

20 g; distilled water 1,000 ml) medium and were transferred to respective slant medium and preserved at 4^{o}C for further study. Isolates were designated as AAUTf, AAUWt, AAUTj, AAUSh, AAUTl, AAUMl and AAUHn for *teff* dough, wheat dough, *tej*, *shamita*, *tella*, molasses and honey respectively.

3.2.2. Preliminary screening of yeasts

Yeast isolates isolated from various sources were grown in YEPD broth (yeast extract, 10 g; peptone, 20 g; glucose, 20 g; distilled water 1,000 ml) for screening on preliminary basis of cell mass and carbon dioxide production. Microbial Cells was detected by measuring optical density at 600 nm using spectrophotometer (Scott, 2011). Isolates with maximum/ heavy biomass were selected for subsequent studies.

Fermentation of carbohydrate or carbon dioxide production was detected by using the method of Barnett *et al*. (2000). The fermentative ability to metabolize carbohydrate was assessed by looking for the formation of gas (CO_2) in durum tubes. Test tubes of about 150mm by 12mm with inserted durum tubes of about 50mm by 6mm were used. The tubes were filled with 10ml of YEPD broth (yeast extract, 10g; peptone, 20g; glucose, 20g; distilled water 1000ml). Each tube was inoculated with 1ml (~1.2x10^{8} cfu/ml) of yeast cell, taken from an actively growing culture. The tubes were incubated at 25^{o}C for about 1 week and examined for bubbles of gas in the inserted tubes (Barnett *et al.*, 2000).

3.3. Identification and characterization of yeasts

3.3.1. Morphological and Cellular characterization

Yeast isolates were identified phenotypically on the basis of colony shape, color, margin, elevation and surface on YEPDA (yeast extract peptone dextrose agar) medium and photographed under a phase contrast microscope Barnett *et al.* (2000) and Yarrow (1998). Preliminary characterization by simple staining (using lactophenol cotton blue dye) of each of the isolates was done (Tankeshwar, 2014). Briefly, isolates were picked and suspended in a drop of phenol cotton blue solution on microscope slide. The slides were then covered with cover slips and observed under a light microscope (B-350 OPTICA, Italy), at × 100 magnifications.

3.3.2. Biochemical Characterization

Biochemical characterization based on the ability of yeast isolates to utilize various carbon sugars were carried out using BIOLOG Yeast MicroPlates. All the isolates were prepared according to the manufacturer's instructions in the OmniLog ID System User Guide (Biolog, Hayward, CA). Briefly, the yeast isolates were sub cultured to Biolog Universal Yeast (BUY) agar and incubated at 26^{o}C for 24 to 48 hours. Yeast cells were transferred aseptically using sterile cotton swabs from the Petri plates into sterile distilled water in sterile glass screw cap test tubes (inoculum density adjusted to 47 ± 2 % transmittance, using the Biolog turbidimeter). The resulting suspension was transferred into a sterile pipette reservoir, and 100 µl was pipetted and inoculated aseptically into each of the 96 wells of the BioLog Yeast MicroPlates and incubated at 26^{o}C. Each YT Micro Plate was read by the Micro Station Reader (BiologInc) at 24, 48, and 72 hours at a single wavelength of 590 nm and were compared with the yeast database (Kurtzman and Fell, 2006).

The Biolog software micro log3 ver. 4.20.05 compared the results obtained with the test strain to the database and provided identification based on distance value of match and separation score produced similarity index value and probability. Acceptable species identification must have similarity index value ≥ 0.5 and probability ≥ 75% were chosen only for species identification and characterization. (Biolog, 1993).

3.3.3. Molecular Characterization

3.3.3.1.DNA Extraction for PCR

DNA extraction of the 23 screened yeast cells was done according to Suh *et al.* (2008). The 500 µl overnight cell suspensions the yeast isolates were transferred into a 1.5 ml Eppendorf tube. The cells were rinsed with 300 µl of TE buffer, vortexed vigorously for 20 sec. followed by centrifugation at 6500 rpm for 10 min at 21^{o}C to pellet cells and the supernatant carefully discarded. Cells were preserved overnight in deep freeze (-20^{o}C) and added 300 µl of TE buffer and incubated at 65^{o}C for 30 min. After incubation, it was frozen at -20^{o}C for 12 hours. Then 50 µl of SDS (sodium dodecyl sulfate) (10-20%) was added and incubated, at 65^{o}C for 45 min. Purification was done by addition of 600 µl of phenol-chloroform, vortexed for 5 min followed

by centrifugation at 10000 for 10 min. Tubes were spine down and the upper layer was transferred to fresh tube.

About 500 µl of isopropanol and 20 µl 3M sodium acetate was pipetted into the tube containing upper solution, and mixed gently by shaking it upside down several times, and the solution was carefully discarded by draining the tube on a clean piece of absorbent paper. The pellet was rinsed with 700 µl 70% EtOH, and incubated at room temperature for 5 min and spin down to wash DNA pellet, and the supernatant (EtOH) was discarded and the tube was drained on a clean piece of absorbent paper, and then air dried for 5 min.

One hundred µl DNA hydration solutions was added and mixed by vortex for 5 sec at medium speed, 1.5 µl RNAse solution was added and again mixed by vortex followed by pulse spinning to collect liquid. It was then incubated at 37^{o}C for 30 min followed by further incubation at 65^{o}C for 1 hour to dissolve the DNA. The samples were incubated at room temperature overnight with gentle shaking upon which the presence of DNA was checked on 1% agarose and visualized under ultraviolet by staining with ethidium bromide. The remaining volume was stored at minus 20^{o}C. The genomic DNA was used as template for subsequent PCR amplifications.

3.3.3.2.PCR amplification of 5.8S-ITS rDNA

Internal transcribed spacer regions (ITS1 and ITS2) and 5.8 S of ribosomal DNA was amplified according to the method described by White *et al.* (1990). The reaction mixture for PCR amplification for all the isolates was prepared using 50 µl volume containing 1 µl ITS-1 (5'-TCC GTA GGT GAA CCT GCG G-3'), 1 µl ITS-4 (5'-TCC TCC GCT TAT TGA TAT GC-3'), 1.0 µl template, 5 µl PCR buffer, 5 µl dNTPs, 1.0 µl polymerase and 36 µl PCR water. The PCR conditions were: 35 cycles including an initial denaturation at 95^{o}C for 10 min, denaturation at 95^{o}C for 30 sec, annealing at 55^{0}C for 1 min and extension at 72^{o}C for 1 min followed by final extension at 72^{o}C for 5 min and holding at 8^{o}C. PCR products were separated on a 1% agarose gel containing 5 µl of ethidium bromide and visualized under UV light. The sizes of the PCR products were estimated by comparing them to a 100 bp Gel pilot DNA molecular weight marker (Qiagen, USA). Negative control reactions without any template DNA were carried out simultaneously.

3.3.3.3.Purification of samples for sequencing

Samples on filter paper were eluted with TE (1mM Tris, 0.1mM EDTA) and amplified with PCR reaction at 95^{o}C 3 min, 46^{o}C for 15 sec, 72^{o}C 30 sec, 95^{o}C for 20 sec x 30 rounds. The products were run on 1.5 % agrose gel in TAE and the amplified bands were cut from the gel, purified using Qiagen Sequencing Purification kit according to manufacturer`s instructions (Qiagen, USA). PGEM®-T Vector was used to clone the rDNA regions, plated onto LB/amp, and performed minipreps on 3 clones from each plate. The sequencing was done by Genewiz sequencing facility service

3.4. Phylogenetic Analysis

The Internal transcribed spacer regions (ITS1 and ITS2) and 5.8 S of ribosomal DNA sequences of isolates were used for a Basic Alignment Search Tools (BLAST). The ITS sequence of the isolates were further aligned and compared to published ITS sequences using the taxonomy browser of the National Center for Biotechnology Information (NCBI). Blasted sequences aligned using ClustalX version 2.0 (Larkin *et al*., 2007). Phylogenetic tree was constructed with molecular evolutionary genetic analysis (MEGA), version 7.0 using a Maximum Likelihood (ML) algorithm character estimation method with bootstrap analyses where 1000 replicates were performed (Kumar *et al*., 2016).

3.5. Screening yeasts for bread fermentation qualities

Further screening of the 23 selected (on the basis of their heavy cell biomass and CO_2 production) isolates was evaluated in terms of dough fermenting ability. The leavening ability of each isolates was evaluated as described by Zaky and Nasr (2011). The following ingredients were mixed; 50 g flour, 0.5 g of better performing yeast isolates, 0.2 g of table sugar, 40 ml of distil water. The dough was then transferred into measuring cylinder (250 ml) and incubated at ambient temperature. The rising power of the yeasts was determined by recording the dough volume after one and two hours.

3.6. Optimization of cultivation condition for yeast growth

3.6.1. Effect of pH on yeast growth

yeasts and control were separately cultured in YEPD (yeast extract peptone Dextrose) broth of pH 3.5, 4, 4.5, 5 and 5.5 and incubated at 30^{o}C for 48 hours (Qureshi *et al.*, 2007) being shaken at 120 rpm. Two flasks each containing 50 ml broth for the listed pH values were inoculated with 48 hours old of yeasts culture 1 ml (approximately 1.2 x10^{8}cells mL^{-1}) separately. Optical densities at 600nm were determined using a spectrophotometer (UV-VIS spectrophotometer, USA) as a measure of growth. The medium yeasts grew was used as blank.

3.6.2. Effect of temperature on yeast growth

The ability of the isolates to grow at different temperature values was examined by inoculating each in duplicate into 50ml YEPD (yeast extract peptone dextrose) broth medium containing yeast extract 1.0%, peptone 2.0%, Dextrose 2.0%. The experiment was arranged at four different temperatures (25, 30, 35, and 40^{o}C) and constant pH 5.5 (result of this study), inoculated with the same number of actively grown yeast cells (48 hours old), 1ml (approximately 1.2 x10^{8}cells mL^{-1}). After 48 hours of incubation optical densities at 600nm were determined using a spectrophotometer (UV-VIS spectrophotometer, USA).

3.6.3. Determination optimum length of time for yeast growth

The optimum time of incubation for a maximum cell biomass production of each yeast isolate was determined by incubating at optimum temperature 30^{o}C (result of this study) for 24, 48, 72, 96 and 120 hours of incubation. The same number of actively yeast cells grown in YEPD for 48 hours, 1ml (approximately 1.2 x10^{8}cells mL^{-1}) was inoculated in duplicates in 50 ml YEPD (yeast extract 1.0%, peptone 2.0%, Dextrose 2.0%) broth in 250ml flask. The best incubation time for growth and maximum biomass production was detected by measuring optical density at 600nm using a spectrophotometer (UV-VIS spectrophotometer, USA).

3.6.4. The interaction effect of temperature, pH and incubation time on yeast growth

The 48 hours old yeast (30^{o}C, 120rpm), cultured in YEPD broth were inoculated with same number of actively grown yeast cells 1ml (approximately 1.2 x10^{8} cells mL^{-1}) at five pH levels (3.5, 4, 4.5, 5 and 5.5) and incubated at 25, 30, 35 and 40^{o}C being shaken at 120 rpm for five

days and samples were taken and analyzed at interval of 24, 48, 72, 96 and 120. Optimum temperature, pH and incubation time for yeast growth and maximum biomass production were determined by using spectrophotometer at 600nm (UV-VIS spectrophotometer, USA).

3.7. Test of hydrogen sulfide production

To examine the capacity to produce H_2S (associated with an off-flavor and unpleasant taste), identified strains were streak cultured on Bismuth Sulphate Agar (BSA) plates and incubated at 30^{o}C for 2 days. Colonies that exhibited much black color along the line of inoculation on BSA plates indicated hydrogen sulfide production (Jiranek *et al.*, 1995). Positive strains were discarded.

3.8. Preparation of wheat bread with selected yeast isolates

3.8.1. Analysis of bread leavening potential of selected yeasts

Bread dough was prepared with potential isolate to observe the baking potency according to Zaky and Nasr (2011). Selected yeasts species for dough making were grown in YEPD broth for 48 hours at optimum temperature of 30^{o}C being shaken at 120rpm. Samples (10 ml each) were centrifuged for 10 min at 5,000 rpm, washed twice with deionized water. The supernatant was discarded. The sedmented yeast biomass with moisture was transferred to pre weighed filter paper and dried overnight at 60^{o}C and stored in a desiccator until constant weight gain (Tamene and Dawit, 2014). The yeast culture was harvested and weighed using analytical balance (FA2104, China).

The prepared dough contained wheat flour (50 g), harvested yeast culture (0.5 g), table sugar (0.2 g) and distilled water (40 ml) in 250 ml measuring cylinders. All the ingredients were properly mixed. Baker's yeast (Saf- instant, from Turkey) was used separately as positive control 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 ambient (24^{o}C) and 30^{o}C temperature for 3 hours. The dough volume was determined by measuring the mean of volume increment at every 30 min interval for 3 hours. All dough samples were plugged using aluminum foil.

3.8.2. Formulation of mixed culture and testing bread leavening potential

The effect of combined (mixed) yeast culture on leavening activity was evaluated. The ingredients used for the dough preparation were wheat flour (50 g), harvested yeast culture (0.5 g), table sugar (0.2 g) and distilled water (40 ml). The dough mixed homogenously and incubated at optimum temperature of 30^{o}C (based on previous result of this study). Single (pure) and mixed of yeast cultures are listed in (Table 4). Two replications were performed for each type of dough fermentation. The rising power of the combined yeasts was determined by recording the dough volume increment starting from zero to two hours at 30 min interval. Aluminum foil was used to plug the dough containing measuring cylinder.

Table 4. Formulation for bread dough preparation.

Mixed culture	Harvested yeast culture in gram	Wheat flour in gram	Table sugar	dH_2O in ml
X1	0.5	0.5	0.2	40
X2	0.5	0.5	0.2	40
X3	0.5	0.5	0.2	40
X4	0.5	0.5	0.2	40
X5	0.5	0.5	0.2	40
X1+X2	0.25 + 0.25= 0.5	0.5	0.2	40
X1+X3	0.25 + 0.25= 0.5	0.5	0.2	40
X1+X4	0.25 + 0.25= 0.5	0.5	0.2	40
X2+X3	0.25 + 0.25= 0.5	0.5	0.2	40
X2+X4	0.25 + 0.25= 0.5	0.5	0.2	40
X3+X4	0.25 + 0.25= 0.5	0.5	0.2	40
X1+X2+X3	0.167+0.167+0.167= 0.5	0.5	0.2	40
X2+X3+X4	0.167+0.167+0.167= 0.5	0.5	0.2	40
X1+X3+X4	0.167+0.167+0.167= 0.5	0.5	0.2	40
X1+X2+X3+X4	0.125+0.125+0.125+0.125= 0.5	0.5	0.2	40

Note:X1-AAUTf1, X2-AAUTf5, X3-AAUTj15, X4-AAUSh17, X5- +ve control, X1+X2-AAUTf1 and AAUTf5, X1+X3-AAUTf1 and AAUTj15, X1+X4-AAUTf1 and AAUSh17, X2+X3-AAUTf5 and AAUTj15, X2+X4-AAUTf5 and AAUSh17, X3+X4-AAUTj15 and AAUSh17), X1 + X2 +X3-AAUTf1, AAUTf5 and AAUTj15, X2+X3+X4-AAUTf5, AAUTj15 and AAUSh17, X1 + X3 + X4-AAUTf1, AAUTj15 and AAUSh17), X1+X2+X3+X4(AAUTf1, AAUTf5, AAUTj15 and AAUSh17)

3.9. Statistical analysis of the experiments

The analyses of variance (ANOVA) of the different sets of experiment or combinations were done using R software version 3.3.1 (de Mendiburu, 2015; R core team, 2016). The mean comparison was made by least significant difference (LSD) at 5% significant level.

4. RESULTS

4.1. Isolation, screening and microscopic observation of yeasts

4.1.1. Isolation and morphological characterization of yeasts

A total of 180 yeast isolates from the samples were isolated of which, 52 isolates and a control with high biomass yield (ranging from 1.7 to 2.6) optical density reading at 48 hours of incubation time (Table 5) selected for further studies. From these, 23 isolates including a control that had higher formation of gas (CO_2) in durum tube within 12 to 24 hours were selected for further analysis (Table 6).

Table 5: Screening of yeast isolates on the bases of biomass yield measured by optical density at 600nm.

Level	Biomass yield	No of isolates
Heavy	1.7-2.6	52
Medium	1.3-1.69	111
Low	0.5-1.29	17
Control	1.7-2.6	1

Table 6: Screening of yeast isolates on the bases of carbon dioxide production

Level	Carbon dioxide production/ hours	Durum tube	No of isolates
Strong	12-24	Empty	23
Medium	>24-48	¾ empty	26
Week	>48	½ empty	3
Control	12-24	Empty	1

The isolates were grouped into five categories and control based on the morphological characteristics (Table 7). All the colonies were smooth and circular in shape with most being cream in color and five being white cream. A greater percentage (65%) of the isolates had raised elevation but three isolates had convex but three with umbonate elevation. Two isolates had elevation of growth in to media whereas all the isolates had an entire margin (Table 7).

Table 7. Phenotypic characterization of yeast isolates

Group	Colony shape	Colony Color	Margin	Elevation	Isolate
1	Circular	Creamy	Entire	Raised	AAUTf2, AAUTf3, AAUTf5, AAUTf8, AAUTf9, AAU_{Tf11}, AAUTf13, AAUTj15, AAUWt21, AAUHn23
2	Circular	Creamy	Entire	Convex	AAUTf4, AAUTf7, AAUTl18
3	Circular	Creamy	Entire	Umbonate	AAUTf6, AAUTf10, AAUTf12
4	Circular	White creamy	Entire	Raised	AAUTf1, AAUTj14, AAUTj16, AAUSh17, AAUSh22
5	Circular	Cream	Entire	Growth into medium	AAUMl19, AAUMl20
6	Circular	Creamy	Entire	Raised	Control

4.1.2. Microscopic observation of selected yeasts

Microscopic observation of the selected 23 showed that isolates AAUTf1-AAUTf13 have circular cellular shape while AAUTj14-AAUHn23 and the control have oval shape with clustered budding (Figure 7).

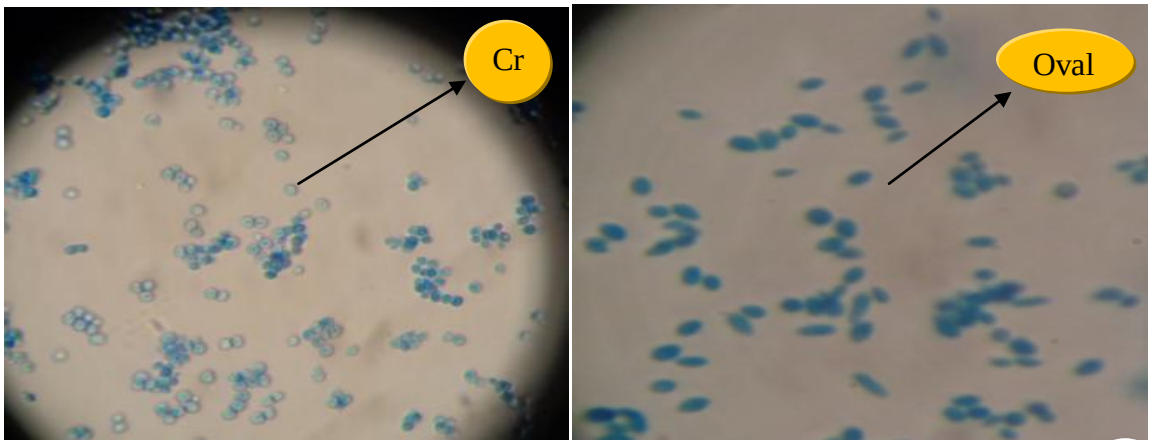


Figure 7: Cellular Morphology of yeasts:
Group I circular includes (AAUTF1 – AAUTF13); Group II oval includes (AAUTJ14-AAUHN23) and control

4.2. Biochemical Characterization of Yeasts

4.2.1. Characterization of yeasts species from oxidation and assimilation test

The twenty three yeast isolates that had high biomass and strong fermentation ability were subjected to biochemical test using Biolog microstation.

4.2.1.1.Utilization test

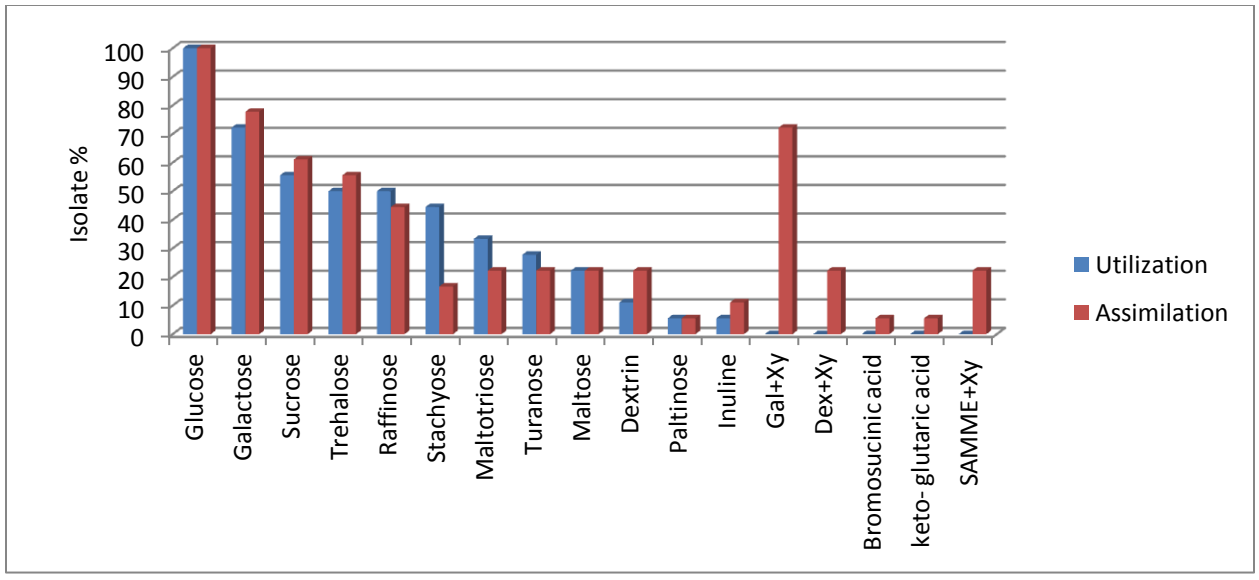
Result of carbon source utilization (the first 1 – 3 column panel of YT Micro Plate) (Appendix 1) indicated that all the isolates oxidized glucose (Table 8). All the isolates except group 5 (AAUTf4, AAUTf6, AAUTf8, AAUTf9, AAUTf10 and AAUTf12) and group 7 (AAUWt22) oxidized sucrose and raffinose in addition to glucose. Maltose and maltotriose were observed being utilized by isolate AAUTj15, AAUSh17, AAUWt21 and AAUTj14 only. Group 1 (AAUTj15, AAUSh17 and AAUWt21), Group 4 (AAUTf5, AAUTf7, AAUTf11 and AAUTf13) and group 5 (AAUTf4, AAUTf6, AAUTf8, AAUTf9, AAUTf10 and AAUTf12) were found capable of oxidized galactose and trehalose and group 3 (AAUTj14) also utilized trehalose. Only AAUTj14 (group 3) utilized Paltinose. The yeast isolates were able to utilize and assimilate 12 and 17carbon sources in that order (Table 8). The percentage of carbon utilization and assimilation of each isolate are described in Figure 8.

4.2.1.2.Assimilation test

The carbon source assimilation of yeast isolates (1-48 column panels) indicated that the assimilation of 17 carbon sources from a total of 60 assimilation profiles (Appendix 1). All yeasts assimilated glucose and followed by galactose (77.8%) and galactose plus xylose (72%). Sucrose, trehalose and raffinose were assimilated by 61%, 55.6% and 44% of the isolates, respectively. Few isolates were found assimilate inulin (5.56%), stachyose (5.56%), palatinose (5.56%), dextrin plus xylose (5.56%), bromo succinic acid (5.56%), a-keto- glutaric acid (5.56%), succinic acid mono-methyl ester plus xylose (5.56%) (Figure 8).

Table 8. Yeast isolates assimilation and oxidation of sugars using Biolog YT Microplate kit.

Group	Isolates	Utilization	Assimilation
1	AAUTj15, AAUSh17 and AAUWt21	Glucose, Sucrose, raffinose, galactose, Maltose, Maltotriose, Turanose	Glucose, Sucrose, ,raffinose, galactose, Maltose, Maltotriose, Turanose, galactose+ xylose
2	AAUTf2 and AAUTf3	Glucose, sucrose, raffinose, dextrin	Glucose, sucrose, raffinose, trehalose, Galactose, Dextrin, Inulin, Dextrin + Xylose, Succinic Acid Mono-Methyl Ester + D-Xylose
3	AAUTj14	Glucose, Sucrose, raffinose, Trehalose Maltose, Maltotriose, Turanose, Stachyose, Palatinose,	Glucose, Sucrose, raffinose, Trehalose Maltose, Maltotriose, Turanose, Stachyose, Palatinose,
4	AAUTf5, AAUTf7, AAUTf11 and AAUTf13	Glucose, Sucrose, raffinose, Trehalose, galactose, Stachyose, Dextrin	Glucose, Sucrose, raffinose, galactose, Trehalose, galactose + D-xylose, Stachyose, Dextrin
5	AAUTf4, AAUTf6, AAUTf8, AAUTf9, AAUTf10 and AAUTf12	Glucose, Trehalose , galactose,	Glucose, Trehalose, galactose, galactose + xylose
6	AAUTl18	Glucose, Sucrose, raffinose, Maltotriose, Turanose Stachyose,	Glucose, Sucrose, raffinose,
7	AAUWt22	Glucose	Glucose, bromo succinic acid, keto- glutaric acid, Succinic Acid Mono-Methyl Ester + D-Xylose



Gal+Xy - Galactose plus Xylose; Dex+Xylose - Dextrose plus Xylose and SAMME+Xy - Succinic acid mono-methyl ester plus Xylose.
Figure 8. Utilization and assimilation of carbon in percent by isolates

4.2.2. Identification of yeast isolates using Biolog microplate kit

The biolog microplate kit identified 18 yeast isolates as 7 species (*Candida humilis, Kluyveromyces lodderae, Saccharomyces boulardi, Schizosacharomyces pombe var malidevora, Schizosacharomyces pombe, Pichia jadini* and *Issatchenika scutulata var exigua*) based on their carbon utilization and assimilations. Dendrogram result indicates that the three isolates $AAU_{Tj15,}$ AAU_{Sh17} and AAU_{Wt21} shared a cluster with 99% similarity and were identified as *Saccharomyces boullardii.* Isolate AAUTf4, AAUTf6, AAUTf8, AAUTf9, AAUTf10 and AAUTf12 were shown belong to *candida humilis* and shared one clade cluster with 99% similarity. *Kluyveromyces lodderae* was found 99% related to isolates AAUTf5, AAUTf11 and AAUTf13 while similar 98% to AAUTf6. Therefore, these isolates were identified as *Kluyveromyces lodderae.* Both isolates AAUTf2 and AAUTf3 were clustered to *Schizosacharomyces pombe var malidevora*. Isolates AAUTj14, AAUTl18 and AAUWt22 were clustered and identified as *Schizosacharomyces pombe, Pichia Jadini* and *Issatchenkia scutulata var exigua* respectively (Figure 9).

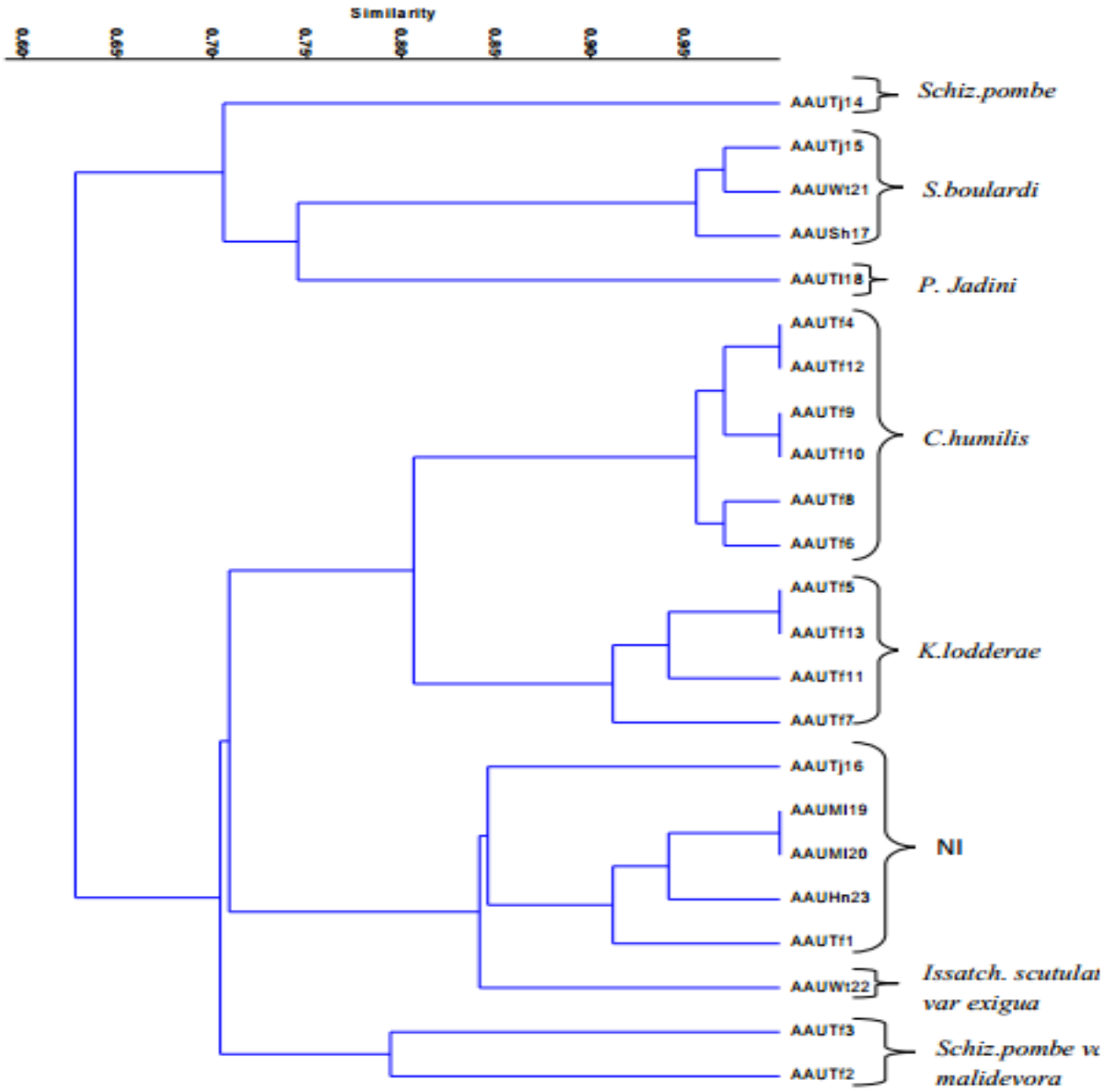


Figure 9. Dendrogram for isolates identified using Biolog kit.
NI: not identified

4.3. Molecular Characterization

In order to identify and determine the correct phylogenetic position of the yeast isolates, molecular genetics identifications were performed. The genomic DNA was extracted from each of the 23 selected isolates, and ITS (none conserved) region including 5.8S which is conserved region of each was amplified with primers ITS1 and ITS4 and sequenced. As shown in Figure 10, PCR products showed a unique variation for the different species analyzed, for example, 444

and 508 bp for *p. fermentans*, 651 bp for *C. humilis*, 726 bp for *K. bulderi*, 935 and 968 bp of *S. cerevisiae* and 587 for *P. kudriavzevii*.

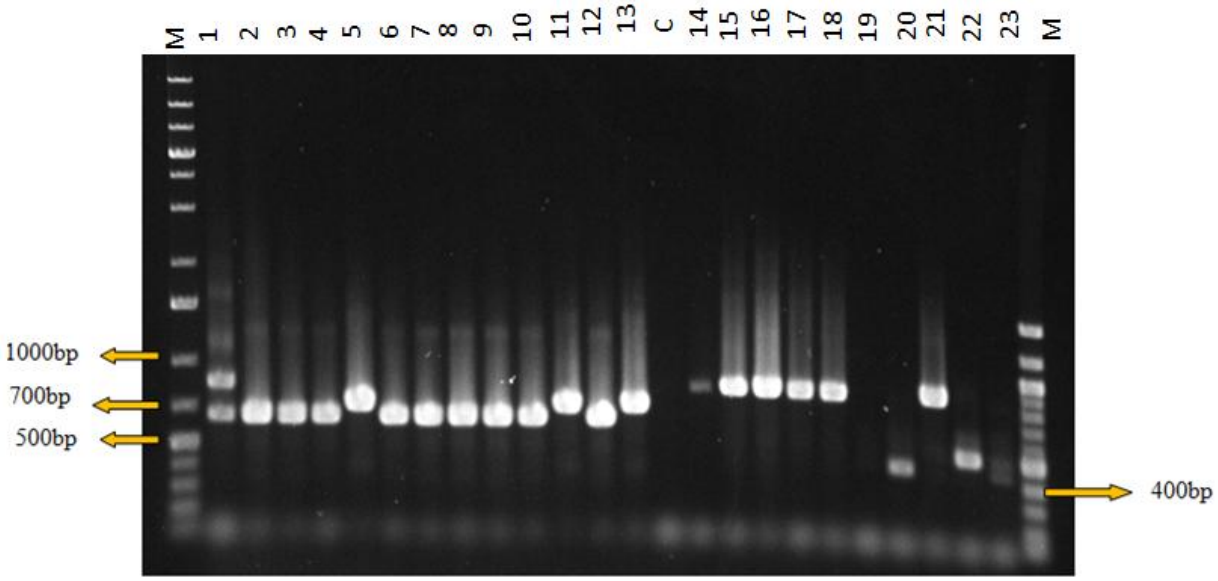


Figure 10. Amplified DNA of 5.8 S ITS DNA of 23 isolates and control run in 1% (W/V) of agarose gel.

Lanes: M, 100bp marker; C, negative control; 1. AAUTf1; 2. AAUTf2; 3. AAUTf3; 4. AAUTf4; 5. AAUTf5; 6. AAUTf6; 7. AAUTf7; 8. AAUTf8; 9. AAUTf9; 10. AAUTf10; 11. AAUTf11; 12. AAUTf12; 13. AAUTf13; 14. AAUTj14; 15, AAUTj15; 16. AAUTj16; 17. AAUSh17; 18, AAUTl18; 19. AAUMl19; 20. AAUMl20; 21. AAUWt21; 22. AAUWt22 and 23. AAUHn23.

The obtained sequence data for the yeast isolates were compared with the sequences of 5.8S-ITS rDNA region available in Genbank for each isolate using BLAST search of the National Center for Biotechnology Information (NCBI) databases. Alignment of the 5.8S-ITS rDNA gene sequences of these isolates with sequences obtained from BLAST search showed different level of similarity to different yeast species. Accordingly, it was found out that all *teff* samples (AAUTf1, AAUTf3, AAUTf4, AAUTf6, AAUTf7, AAUTf8, AAUTf9, AAUTf10, AAUTf11 and AAUTf12) had 99% similarity with *Candida humilis*, except AAUTf5 and AAUTf13 were 99% closely related to *Kazachstania bulderi*. Similarly, AAUTj15, AAUSh17 and AAUMl20 showed 99% similarity with *Saccharomyces cerevisiae*, *Saccharomyces cerevisiae* and *Pichia fermentans*, respectively. Isolate AAUWt21 (97% homology) and AAUHn23 (98% homology) showed closest similarity to *Pichia kudriavzevii* and *Pichia fermentans* respectively (Table 9).

Table 9. Identification of yeast isolates by comparative sequence of the 5.8S-ITS rDNA region.

Isolate	Sample type	Identity (%)	Closest Relative	Accession number
AAUTf1, AAUTf3, AAUTf4, AAUTf6, AAUTf7, AAUTf8, AAUTf9, AAUTf10, AAUTf11 and AAUTf12	*Teff dough*	99	*Candida humilis*	KY102138.1(CBS)
AAUTf5 and AAUTf13	*Teff dough*	99	*Kazachstania bulderi*	KY103628.1 (CBS)
AAUTj15	*Tej*	99	*Saccharomyces cerevisiae*	KY105143.1 (CBS)
AAUSh17	*Shamita*	99	*Saccharomyces cerevisiae*	KY630581.1(GITA)
AAUMl20	Molasses	99	*Pichia fermentans*	KY104550.1 (CBS)
AAUWt21	Wheat dough	97	*Pichia kudriavzevii*	KY104596.1(CBS)
AAUHn23	Honey	98	*Pichia fermentans*	KY104543.1(CBS)

4.3.1. Phylogenetic Analysis of the 5.8S-ITS rDNA Sequences

Phylogenetic analysis demonstrated that the isolates belonged to five species; *Candida humilis, Kazachstania bulderi, Saccharomyces cerevisiae, Pichia fermentans* and *Pichia kudriavzevii* (Figure 10).

The phylogenetic tree indicated that the isolates AAUTf1, AAUTf3, AAUTf4, AAUTf6, AAUTf7, AAUTf8, AAUTf9, AAUTf10, AAUTf11, AAUTf12 and *Candida humilis* shared one clade cluster with similarities 99 %. Therefore, these isolates were identified as *C. humilis*. Isolates AAUTf5 and AAUTf13 and *Kazachstania bulderi* shared one clade cluster with the similarity of 100% and was thus identified as *K. bulderi*. BLAST results revealed that the two isolates AAUTj15, AAUSh17 were found sharing one clade cluster with 100% similarity to *Saccharomyces cerevisiae*. Therefore, AAUTj15 and AAUSh17 were identified as *S. cerevisiae*.

Isolates AAUMl20 and AAUHn23 were closely related to *P. fermentans* with 99% similarity and thus were identified as *P. fermentans*. Isolate AAUWt21 was identified as *P. kudriavzevi* as a result of sharing of a clade cluster with 99% similarity (Figure 11).

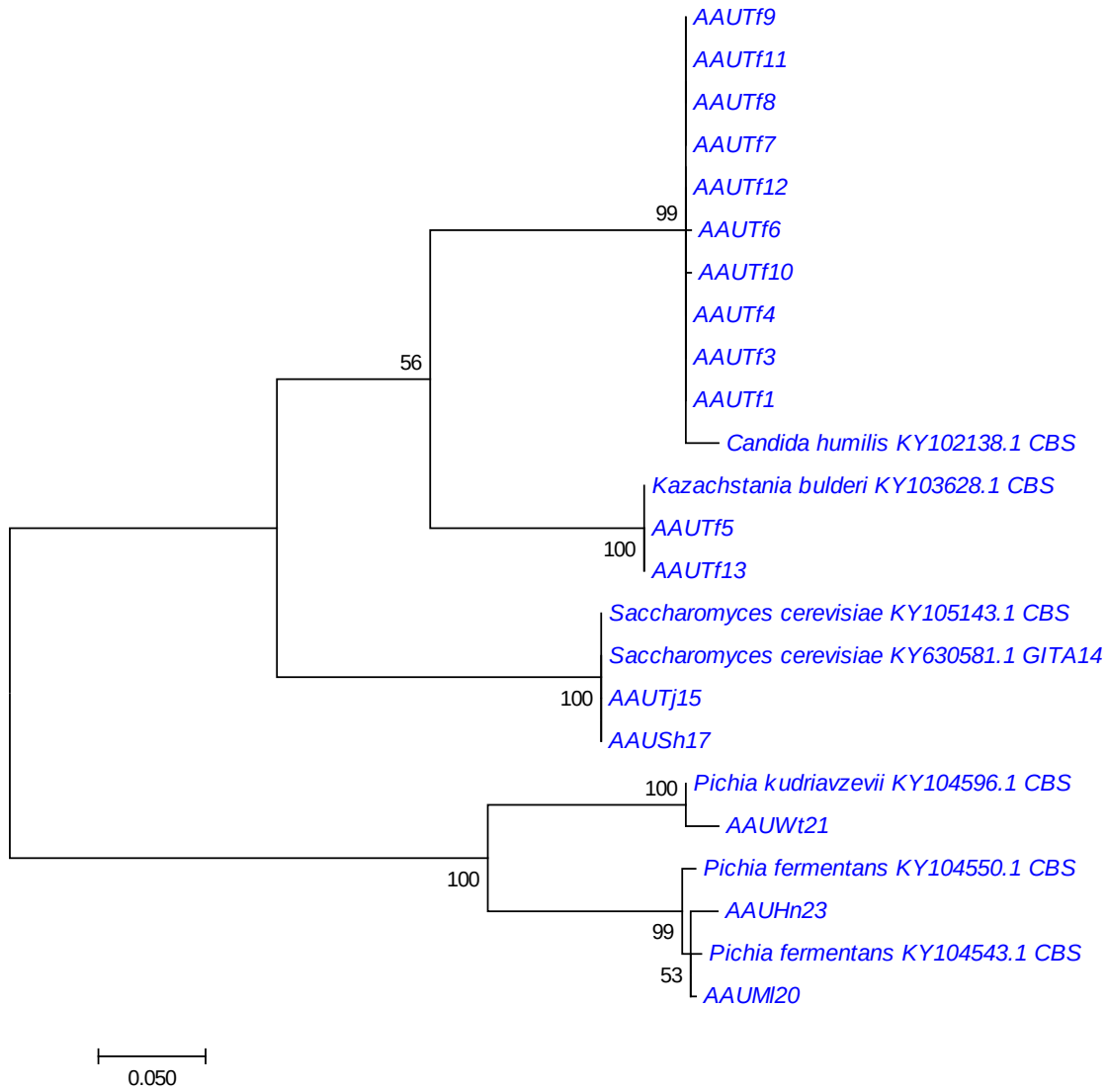


Figure 11. Phylogenetic tree of ITS sequences using MEGA 7 software with 1000 bootstrap value was used to draw the tree. GenBank accession numbers follow species names.

4.4. Comparison of conventional, biolog and molecular methods for the identification of yeasts

Comparison of conventional, biolog and molecular methods for the identification of yeasts is presented in Table 10. In this study, 4 isolates (AAUTf4, AAUTf6, AAUTf10 and AAUTf12) as *Candida* and 2 isolates (AAUTj15 and AAUSh17) as *Saccharomyces* were identified to genus level with all the three techniques. In this result indicated that of the 18 (78.3%) identified isolates, the identification of 6 (33%) isolates (AAUTf4, AAUTf6, AAUTf8, AAUTf9 AAUTf10 and AAUTf12) with both biochemical and molecular methods was found matching at species level; and the identification of 3 (AAUTf5, AAUTf11 and AAUTf13) were identified the same to genus level by the method of conventional and biochemical.

Table 10. Comparison of Conventional, biochemical and molecular yeast identification techniques

Strain	Conventional (Genus)	Microplate YT (Biolog)	Molecular techniques
AAUTf1	*Saccharomyces*	Not identified	*Candida humilis*
AAUTf2	*Kluyveromyces*	*Schizosacharomyces pombe var malidevora*	Not identified
AAUTf3	*Kluyveromyces*	*Schizosacharomyces pombe var malidevora*	*Candida humilis*
AAUTf4	*Candida*	*Candida humilis*	*Candida humilis*
AAUTf5	*Kluyveromyces*	*Kluyveromyces lodderae*	*Kazachstania bulderi*
AAUTf6	*Candida*	*Candida humilis*	*Candida humilis*
AAUTf7	*Candida*	*Kluyveromyces lodderae*	*Candida humilis*
AAUTf8	*Kluyveromyces*	*Candida humilis*	*Candida humilis*
AAUTf9	*Kluyveromyces*	*Candida humilis*	*Candida humilis*
AAUTf10	*Candida*	*Candida humilis*	*Candida humilis*
AAUTf11	*Kluyveromyces*	*Kluyveromyces lodderae*	*Candida humilis*
AAUTf12	*Candida*	*Candida humilis*	*Candida humilis*
AAUTf13	*Kluyveromyces*	*Kluyveromyces lodderae*	*Kazachstania bulderi*
AAUTj14	*Saccharomyces*	*Schizosacharomyces pombe*	Not identified
AAUTj15	*Saccharomyces*	*Saccharomyces boullardii*	*Saccharomyces cerevisiae*
AAUTj16	*Saccharomyces*	Not identified	Not identified
AAUSh17	*Saccharomyces*	*Saccharomyces boullardii*	*Saccharomyces cerevisiae*
AAUTl18	*Candida*	*Pichia Jadini*	Not identified
AAUMl19	Not identified	Not identified	Not identified
AAUM20	Not identified	Not identified	*Pichia fermentans*
AAUWt21	*Kluyveromyces*	*Saccharomyces boullardii*	*Pichia kudriavzevii*
AAUWt22	*Saccharomyces*	*Issatchenkia scutulata var exigua*	Not identified
AAUHn23	*Kluyveromyces*	Not identified	*Pichia fermentans*

4.5. Selection of yeast isolates for bread fermentation

The leavening capacity of the 23 isolates was evaluated, thus six isolates showed good performance of fermentation and selected for further study (Table 11).

Table 11. Performance evaluation of yeast isolates on leavening capacity.

Yeast isolates	Leavening volume (cm3)		Volume increment (cm3)	Time required (Hours)
	initial	final		
AAU_{Tf1}	78	164	86	4
AAU_{Tf2}	78	148	70	6
AAU_{Tf3}	78	156	78	6
AAU_{Tf4}	78	132	54	6
AAU_{Tf5}	78	160	82	4
AAU_{Tf6}	78	146	68	8
AAU_{Tf7}	78	168	90	8
AAU_{Tf8}	78	152	74	8
AAU_{Tf9}	78	134	56	7
AAU_{Tf10}	78	144	66	7
AAU_{Tf11}	78	170	92	6
AAU_{Tf12}	78	174	96	8
AAU_{Tf13}	78	178	100	8
AAU_{Tj14}	78	190	112	8
AAU_{Tj15}	78	191	113	3
AAU_{Tj16}	78	180	102	6
AAU_{Sh17}	78	195	118	3
AAU_{Tl18}	78	166	88	6
AAU_{M19}	78	110	32	8
AAU_{Ml20}	78	138	60	5
AAU_{Wt21}	78	151	73	5
AAU_{Wt22}	78	170	92	6
AAU_{Hn23}	78	116	48	8

4.6. Optimization of cultivation conditions for yeast growth

4.6.1. The effect of pH on yeast biomass

The growth of the isolates at different pH values was observed to vary (Table 12). All the isolates were able to grow at all pH levels. The minimum and maximum growth yield was observed at pH 3.5 and 5.5 values, respectively. The maximum biomass yields at pH 5.5 for isolate AAUMl20, AAUSh17, AAUWt21 and AAUTj15 were 2.57, 2.45, 2.25 and 2.23

respectively. Isolate AAUM120 was found to gain the highest biomass yield at the same pH value. However, the maximum biomass (1.844) for the control was achieved at pH 5. Biomass of each isolate detected at 600nm OD. There were significant ($p < 0.05$) differences among the biomass yield of the isolates at each pH values (Table 12).

Table 12. Mean biomass of Yeasts under different pH ranges

Isolate	pH3.5	pH4	pH4.5	pH5	pH5.5
AAUTf1	0.64^{op}	1.56^{defgh}	1.50^{defghi}	1.82^{bcdef}	1.91^{bcde}
AAUTf5	0.57^{p}	1.43^{fghij}	1.51^{defghi}	1.63^{defgh}	1.85^{bcdef}
AAUTj15	0.76^{mnop}	1.28^{ghijk}	1.2^{hijklm}	1.56^{defgh}	2.23^{abc}
AAUSh17	0.74^{nop}	1.05^{jklmno}	1.2^{hijklm}	1.81^{bcdef}	2.45^{a}
AAUMl20	0.83^{lmnop}	1.09^{ijklmn}	1.25^{ghijkl}	1.47^{efghij}	2.57^{a}
AAUWt21	0.73^{nop}	0.97^{klmnop}	1.68^{defg}	1.94^{bcd}	2.25^{ab}
Control	1.67^{defg}	1.68^{defg}	1.67^{defg}	1.84^{bcdef}	1.77^{cdef}

Note: Means with the same letter are not significantly different at $p < 0.05$.

4.6.2. The Effect of temperature on yeast biomass

The yeast isolates were observed growing at all temperature values (Table 13). The maximum biomass yield for all the six yeast isolates and the control was observed at 30^{o}C and the minimum biomass yield for all the isolates (including the control) was observed above 35^{o}C. Optical density (OD) reading value of 2.6 for AAUMl20 isolate was noted maximum growth at 30^{o}C. The highest growth rates observed for AAUTf1, AAUTf5, AAUTj15, AAUSh17, AAUWt21 and control isolates were 1.9, 1.87, 2.21, 2.39, 2.27 and 1.79 OD at 600nm, respectively. The biomass yield of all the isolates was found significantly higher at 30^{o}C than at other temperature values (25^{o}C, 35^{o}C or 40^{o}C). The biomass yield for the control did not show significant difference ($p>0.05$), at temperature 30 and 35^{o}C (Table 12). The minimum biomass yield observed was 0.47, 0.4, 0.46, 0.52, 0.53, 0.52 and 1.11, for isolates AAUTf1, AAUTf5, AAUTj15, AAUSh17, AAUMl20, AAUWt21 and control in that order. The optimum temperature for the yeast isolates recorded in this study was found at 30^{o}C (Table 13).

Table 13. Mean biomass of potent yeasts under different temperature ranges

Isolate	**25°C**	**30°C**	**35°C**	**40°C**
AAUTf1	0.6^{hijk}	1.9^{d}	0.56^{hijk}	0.47^{jk}
AAUTf5	0.61^{hij}	1.87^{de}	0.58^{hijk}	0.40^{k}
AAUTj15	0.56^{hijk}	2.21^{c}	0.59^{hijk}	0.46^{jk}
AAUSh17	0.64^{hij}	2.39^{ab}	0.72^{h}	0.52^{hijk}
AAUMl20	0.67^{hi}	2.6^{a}	0.69^{hi}	0.53^{hijk}
AAUWt21	0.51^{ijk}	2.27^{bc}	0.57^{hijk}	0.52^{ijk}
control	1.42^{f}	1.79^{de}	1.63^{e}	1.11^{g}

Note: Means with the same letter are not significantly different at $p<0.05$.

4.6.3. Incubation period on yeast biomass yield

The effect of incubation time on the growth rates of the six isolates and the control at optimum temperature (30°C) and pH of 5.5 is shown in Table 14. Maximum biomass yield was obtained for all the yeast isolates of this study at 48 hours but the minimum biomass yield was recorded decreasing then after to the minimum level at 120 hours. Isolate AAUMl20 achieved the highest biomass yield (2.57) at 48 hours of incubation time and optimum temperature 30°C followed by isolate AAUSh17 (2.41 OD_{600nm}) with similar incubation time and temperature. However, the control had gained maximum biomass yield at 72 hours of incubation time with OD value of 1.993.

Minimum biomass yield of 0.52, 0.61, 0.43, 0.43 and 0.53 OD values were observed for isolates the AAUTf5, AAUTj15, AAUSh17, AAUMl20, and AAUWt21 at 120 hours of incubation. Regarding this result, the best incubation time for the yeast isolates growth was found to be 48 hours except for the control that showed the highest biomass at 72 hours (Table 14).

Table 14. Mean growth of Yeasts under different incubation time ranges

Isolate	24hours	48hours	72hours	96hours	120hours
AAUTf1	1.6^{gh}	1.96	1.05^{jk}	0.59^{o}	0.36^{q}
AAUTf5	1.49^{h}	1.81^{ef}	0.79^{n}	0.94^{klm}	0.52^{op}
AAUTj15	1.68^{efg}	2.12^{cd}	1.33^{i}	0.84^{mn}	0.61^{o}
AAUSh17	1.79^{ef}	2.43^{b}	0.91^{lmn}	0.81^{mn}	0.43^{pq}
AAUMl20	1.7^{efg}	2.6^{a}	0.99^{kl}	0.83^{mn}	0.43^{pq}
AAUWt21	1.66^{fg}	2.21^{c}	1.15^{j}	0.98^{kl}	0.53^{op}
Control	1.31^{i}	1.74^{efg}	1.99^{cd}	1.81^{e}	1.8^{e}

Note: means with the same letter are not significantly different at $p<0.05$.

4.6.4. Combined effect of temperature, pH and incubation time on yeast biomass yield

The maximum cell density 1.89, 1.82, 2.17, 2.41, 2.56 and 2.23 of OD at 600nm for isolates AAUTf1, AAUTf5, AAUTj15, AAUSh17, AAUMl20 and AAUWt21 respectively, were obtained at 30^{o}C, pH 5.5 and 48 hours of incubation (Appendix 2). On the other hand, the maximum biomass yield for control (2.0, OD_{600nm}) was achieved when the temperature, pH and incubation time was at 30^{o}C, 5 and 72 hours, respectively. There was significant difference ($p< 0.05$) among the treatments on the combined effect of temperature, pH and incubation time with regard to biomass yield. The minimum biomass yield was measured for the isolates AAUTf1 (0.21), AAUTf5 (0.25), AAUTj15 (0.27), AAUSh17 (0.28), AAUMl20 (0.35), AAUWt21 (0.28) and control (0.49, OD_{600nm}) at 40^{o}C, 3.5 pH and 120 hours of incubation time (Appendix 2).

4.7. Hydrogen sulphide production by yeast isolates

On the basis of their H_2S production (Figure 12), the isolates were grouped into three categories (non-producers, light level and heavy level of H_2S producers). Accordingly, isolate AAUTf1 did not produce hydrogen sulfide (Figure 12, A), while AAUTf5, AAUTj15 and AAUSh17 produced light level of hydrogen sulfide. The commercial yeast also produces light level H_2S as well (Figure 12, B). Isolates AAUMl20 and AAUWt21 produced heavy level of hydrogen sulfide (Figure 12, C). Therefore, AAUTf1, AAUTf5, AAUTj15 and AAUSh17 were subjected for further test.

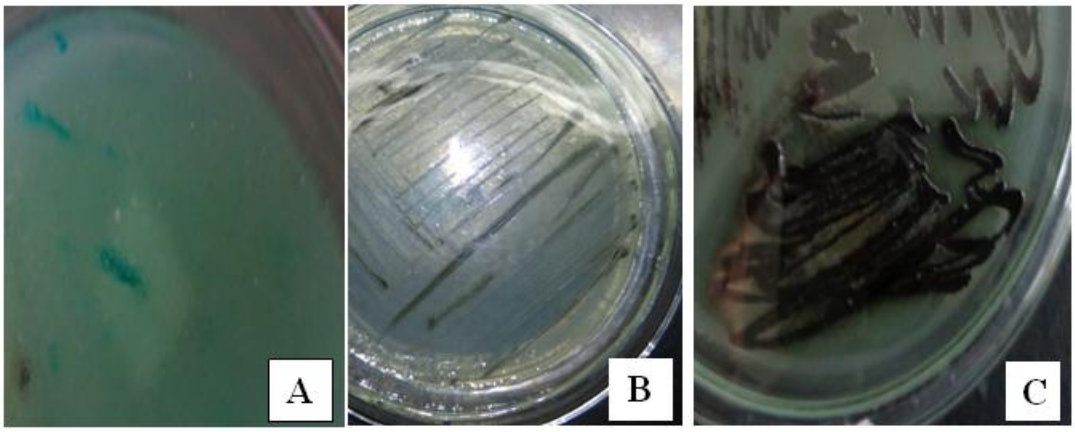


Figure 12. H_2S gas production by isolates:
A (AAUTf1) - non producer; B (AAUTf5, AAUTj15, AAUSh17 and Commercial yeast) - light level and C (AAUMl20 and AAUWt21) - heavy level

4.8. Leavening capacity of yeast strains

None hydrogen sulphide producer; *C. humilis* strain (AAUTf1) and light H_2S producer *K. bulderi* strain (AAUTf5), *S.cerevisiae* strain (AAUTj15), and *S.cerevisie* strain (AAUSh17) (Figure 12) were compared to commercial *S.cerevisiae* based on leavening capacity.

From the result, it was observed that the period of bread dough fermentation, at 30^{o}C was found to be short (2 hours) compared to ambient temperature (Table 14). Maximum mean of leavening activity was exhibited by isolate AAUTf1 (131 cm^3) at 120 min, which was followed by AAUTf5 (128 cm^3) at 120 min at 30^{o}C. Similarly, isolates AAUSh17 (127 cm^3) and AAUTj15 (125 cm^3) achieved high leavening activity, at 60 min at the same temperature, which was not significantly different ($p>0.05$) with the above isolates (AAUTf1 and AAUTf5). The commercial yeasts had 117 cm^3 mean rising capacity, at 90 min which is lower baking activity and longer leavening time ($p<0.05$) than that of the indigenous isolates. Dough left to ferment without yeast (negative control) did not show volume increment within 3 hours of dough fermentation (Table 15).

Table 15. Rising power of potent yeast strains at ambient (24^{o}C) and 30^{o}C temperature

Isolates	Temp.^{o}C	Mean of rising dough volume (cm^3) / Time (min)						
		0	30	60	90	120	150	180
AAUTf1	24	0^{i}	12^{hi}	26^{g-i}	50^{d-i}	63^{a-g}	70^{a-g}	109^{ab}
	30	0^{l}	32^{kl}	46^{i-k}	73^{e-j}	131^{a}	110^{a-e}	84^{c-h}
AAUTf5	24	0^{i}	35^{f-i}	51^{d-h}	75^{a-g}	97^{a-d}	81^{a-f}	70^{a-g}
	30	0^{l}	40^{jk}	$80d^{-i}$	111^{b-d}	128^{a}	124^{ab}	81^{d-i}
AAUTj15	24	0^{i}	60^{b-h}	113^{a}	116^{a}	87^{a-e}	73^{a-g}	67^{a-g}
	30	0^{l}	49^{g-k}	125^{ab}	110^{b-d}	81^{d-i}	84^{c-h}	77^{d-j}
AAUSh17	24	0^{i}	51^{d-h}	55^{c-h}	59^{b-h}	98^{a-d}	86^{a-f}	82^{a-f}
	30	0^{l}	48^{h-k}	127^{a}	103^{b-e}	78^{d-i}	86^{b-g}	63^{f-k}
CY	24	0^{i}	39^{e-i}	90^{a-d}	100^{a-d}	103^{a-c}	80^{a-f}	73^{a-g}
	30	0^{l}	47^{h-k}	101^{b-e}	117^{b-c}	103^{b-e}	95^{b-f}	78^{d-i}
NC	24	0^{i}	0^{i}	0^{i}	0^{i}	0^{i}	0^{i}	0^{i}
	30	0^{l}	0^{l}	0^{l}	0^{l}	0^{l}	0^{l}	0^{l}

NB: CY- commercial yeast; NC – negative control.
Means with the same letter are not significantly different at $p<0.05$.

4.9. Effect of mixed yeast cultures on leavening activity

The combined ability of the four selected yeast isolates AAUTf1 (*C. humilis*), AAUTf5 (*K. bulderi*), AAUTj15 (*S.cerevisie*) and AAUSh17 (*S.cerevisie*) on bread dough leavening was determined (Table 16). Co-inoculated isolates were compared for their leavening effect to each of separate isolate and to that of commercial yeast was evaluated. Result of the three co-inoculated isolates (AAUTf1+ AAUTf5 + AAUTj15) was found the highest (143 cm^3) at 90 min; while the raising volume of dough of as result of co-inoculation of different combination of two (AAUTf5 + AAUTj15) and three (AAUTf1 + AAUTj15 + AAUSh17) yeast isolates was found as high as 141 and 140 cm^3 respectively at 60 min (Table 16). Separate inoculation of single isolate of AAUSh17 and AAUTj15 leavening potential in raising the volume of fermenting dough, at 60min; was found as high as 127 and125 cm^3 of each, in that order.

Table 16. Leavening activity of mixed and pure isolates

Isolate/S	Mean of rising dough volume (cm^3) / Time (min)				
	0	30	60	90	120
X1	0^{B}	32^{zA}	46^{w-A}	73^{n-w}	131^{a-c}
X2	0^{B}	40^{y-A}	80^{l-t}	111^{c-j}	128^{a-d}
X3	0^{B}	49^{v-z}	125^{a-e}	87^{h-t}	81^{k-t}
X4	0^{B}	48^{w-z}	127^{a-d}	93^{g-r}	78^{l-u}
X5	0^{B}	47^{w-A}	101^{d-n}	117^{b-h}	103^{c-m}
X6	0^{B}	0^{B}	0^{B}	0^{B}	0^{B}
X1X2	0^{B}	49^{v-z}	102^{d-m}	68^{q-y}	68^{q-y}
X1X3	0^{B}	44^{x-A}	98^{e-o}	94^{g-r}	83^{j-t}
X1X4	0^{B}	19^{AB}	96^{f-q}	85^{j-t}	66^{r-y}
X2X3	0^{B}	100^{d-n}	141^{ab}	121^{a-h}	86^{i-t}
X2X4	0^{B}	33^{zA}	124^{a-f}	109^{c-k}	63^{s-y}
X3X4	0^{B}	64^{s-y}	114^{b-i}	77^{m-v}	69^{p-x}
X1X2X3	0^{B}	19^{AB}	97^{e-p}	143^{a}	98^{e-o}
X2X3X4	0^{B}	50^{u-z}	128^{a-d}	93^{g-r}	71^{0-x}
X1X3X4	0^{B}	59^{t-z}	140^{ab}	106^{c-l}	89^{h-s}
X1X2X3X4	0^{B}	51^{u-z}	125^{a-e}	109^{c-k}	89^{h-s}

Note: nomination for isolates X1-AAUTf1; X2-AAUTf5; X3-AAUTj15; X4-AAUSh17; X5-CY (Positive control); X6- Negative control.

5. DISCUSSION

The result of the cultural characteristics showed that the colonies of the yeast isolates were creamy, white creamy, circular, smooth, raised, convex, umbonate and growth in to medium (Table 7). Similar findings were also observed by Peu *et al.* (2012), who reported that typical yeast colonies are creamy to white creamy color and circular colony shape. Microscopically, the vegetative cells are oval, circular in clusters and chains with budding (Figure 7). Based on the colony characteristics the selected isolates (AAUTf1, AAUTf5, AAUTj15 and AAUsh17) were found to belong to genus *Sacharomyces, Kluyveromyces* and *Candida* which are unicellular ascomycete as noted by Boekhout and Kurtzman (1996) and Barnet *et al.* (2000). However, it is currently accepted that phenotypic analyses are not sufficient for a trust worthy identification at the species level (Kurtzman and Robnett, 2003; Manzano *et al*., 2004) since phenotypic characters can be influenced by strain diversity and cultivation conditions (Foschino *et al.*, 2004). Therefore, the laboratory protocols provided for each identification method should be compared (Deak and Beuchat 1996; Kurtzman and Fell 1998).

Isolates that belong to genera *Saccharomyces, Candid*a and *Kluyveromyces* were found utilizing carbon sources such as glucose, sucrose, raffinose, trehalose and maltose. Vaughan-Martini and Martini (1998) suggested that for an isolate to be accepted as baker's yeast it must be able to assimilate different carbon sources including glucose, sucrose, maltose and raffinose. This indicates that these isolates fulfill characteristics of baker's yeast. In addition, these genera utilized and assimilated galactose. According to Jimoh *et al*. (2012), galactose is a nonconventional nutrient for yeasts, which however can be used as sole carbon source when glucose is absent from the medium. Thus, the ability of yeast cells to assimilate galactose indicates the expression of gal genes (Balarabe *et al.*, 2017).

On the basis of utilization and assimilation of 94 different carbon sources, the yeast isolates were identified using Microplate YT Biolog (Biolog, USA). The results from Biolog system indicated that the species *Candida humilis* (26.1%) only got identity which is similar to molecular identification at species level and *Sacharomyces cerevisiae* (8.7%) at genus level, while for 65% of the other isolates there was no exact matching (Table 10). Arias *et al*. (2002) have come to a similar conclusion when examining yeasts isolated from orange juice with only 13–35% of yeast

isolates correctly identified by assimilation methods (Biolog). Similarly, Sand and Rennie (1999) have pointed out that mis identification of species belonging to the genus *Candida* frequently occurred using the Biolog and API systems.

The commercial identification kit Microplate YT Biolog, were limited to identify yeasts from the environment (foods and drinks) at species level, probably the data base were designed to meet the needs of clinical diagnostics (Roberto *et al.*, 2004) and also could be associated with the absence of their biochemical properties in the reference library or database used for the identification. The poor identification by assimilation testing may be caused by newly reported yeast species and could be resolved simply by regular updating of new species into assimilation data base (Spencer *et al*., 2011).

Mantynen *et al*. (1999) have reported that misidentification of strain *C. milleri* and most maltose negative yeast isolates from Finnish sourdough to *K. lodderae*. In the same study other yeast isolates, which were ascribed to *Saccharomyces boulardii* by the Biolog system, clustered together with *S. cerevisiae* strains by molecular methods. These observations are very similar to those presented in this work since isolate AAUTj15 and AAUSh17 were identified as *S. boulardii* by Biolog system while to *S. cerevisiae* by molecular method (Table 10).

In recent years, the use of molecular methods for the detection, identification and characterization of microorganisms in foods has attracted a lot of attention, and these methods are currently considered an indispensable tool that permits the accurate description of the microbial ecology of foods and beverages (De Vuyst and Vancanneyt, 2007). Among the molecular methods, amplification by PCR of the region spanning the two intergenic transcribed spacers (ITS) and the 5.8S ribosomal gene (ITS1–5.8SrDNA–ITS2), followed by restriction analysis and sequencing, has been found valuable for differentiation of several yeast species, including *Saccharomyces* spp. (Guillamon *et al*., 1998; Esteve-Zarzoso *et al*., 1999). Sequencing of the internal transcribed spacers (ITS) occurring between the 18S and 28S rDNA genes was investigated in order to confirm the identification of yeasts at species level (Oda *et al*., 1997; Montrocher *et al.,* 1998). Hesham *et al*. (2014) also reported that, 5.8S-ITS rDNA and D1/D2 domain of the 26S rDNA gene analysis is a reliable routine technique for the differentiation of yeasts at species level.

The identity of the isolates based on the homologies of the sequences of the ITS region with those deposited in the Genbank databases is summarized in Table 9. Therefore, the results of the study is consistent with other studies that have reported that molecular methods based on the 5.8S-ITS rDNA region are rapid and precise tools when compared to conventional (physiological, biochemical etc) methods for the identification and typing of yeast species (White *et al*., 1990).

In this study, the amplified ITS-5.8S region showed bands ranging from 444 to 968 bp. Similar to this study, the amplification of yeasts commonly *Candida* species with ITS1 and ITS4 primers yielded fragments 350 to 880 bp long (Fujita *et al*., 2001). Korabecna *et al. (*2003) also reported that, the *candida* and *saccharomyces* species length of amplified fragments using the primers ITS1 and ITS4 were 380-900 respectively. Different yeast species were identified based on ITS-5.8S rDNA sequence analysis that included *Candida humilis* (AAUTf1, AAUTf3, AAUTf4, AAUTf6, AAUTf7, AAUTf8, AAUTf9, AAUTf10, AAUTf11 and AAUTf12), *Kazchistania bulderi (*AAUTf5 and AAUTf13), *Saccharomyce cerevisiea* (AAUTj15 and AAUSh17) and *Pichia fermentans (*AAUMl20), with 99% homology except *Pichia kudriavzevii* (AAUWt21) and *Pichia fermentans* (AAUHn23) which were identified with 97% and 98% homology respectively (Table 9). In consistence to the present study, Ozel *et al*. (2015) showed that, ITS-5.8S rDNA sequence analysis of yeast isolates collected during the tarhana dough fermentation identified yeasts representing different clusters. These yeast isolates displayed higher (98%) similarity to species *Pichia kudriavzevii*, *Candida glabrata*, *Candida humilis*, *Saccharomyces cerevisiae*, *Kluyveromyces marxianus*, *Kazachstania servazzi* and *Kazachstania unispora*. Fernandez-Espinar *et al*. (2000) have also identified flour yeasts using ITS-5.8S rDNA sequence analysis. Similarly, a study by Roberto *et al*. (2004) have also reported that sequences of the internal transcribed spacers region for yeasts isolated from fermented foods (fermented dough) were *Candida, Sacharomyces* and *Pichia* species which is in line to the present study that the ITS region permitted a consistent identification of the yeast isolates to *Candida, Sacharomyces and Pichia*) from fermented foods, beverages and sugar factory byproducts (molasses). Cultural and molecular genetics identification of yeast species originating from different fruits, foods and drinks are dominated by *S. cereviciea*, *C. humilis and Pichia* species (Teramoto *et al.,* 2005; Oslan *et al*., 2012).

The production efficiency of a strain depends on many factors such as temperature, pH, incubation period, inoculums size, nature etc. (Supanwong *et al*., 1983). This study evaluates the optimum temperature, pH and incubation period of the potent yeast isolates. An interesting observation from these data showed that all the isolates, *Candida humilis* (AAUTf1) *Kazachitania bulderi* (AAUTf5), *Saccharomyces cerevisiae* (AAUTj15 and AAUSh17), *Pichia fermentans* (AAUMl20) and *Pichia kudrvizivi* (AAUWt21) showed higher growth at pH of 5.5, temperature of 30^{o}C and incubation time of 48 hours, while the commercial yeast (control) considered in this study observed growing to the maximum mass at pH 5, temperature of 30^{o}C and incubation period of 72 hours.. This shows that the isolated yeasts (this study) had shorter growth time than that of the commercial yeast strain. Similar to this result, Dechassa (2010) have found that yeasts grew to the maximum at pH 5 to 5.5, 30^{o}C temperature and 72 hours of incubation.

All the yeast species (this study) could tolerate a temperature up to 40^{o}C including control (Table 13 and Appendix 2). In agreement with this study, Choi *et al*. (2010); Nitayavardhana *et al*. (2010) also reported that yeasts can grow at elevated temperatures of 40^{o}C, but the optimal temperature is approximately 30^{o}C which is in consistent with the present study. In contrary to the present findings, Ma'aruf *et al*. (2011) observed that yeast isolates could only tolerate a temperature up to 37^{o}C. The ability of yeast to tolerate high temperature suggests that the isolates can withstand excess heat associated with fermentation process and therefore can be used to accomplish fermentation at a wide range of temperature condition.

In this study maximum OD obtained at 48 hours of incubation and decreasing their biomass yield with increasing incubation period. This could be due to the fact that most yeast cells density reduced to the minimum at stationary and death phase after 48 hours of incubation. This is supported by Clara (2015), stationary phase of yeast growth is a period of no growth when metabolism slows and cell division is stopped due to nutrient deprivation, toxic metabolites and high temperatures which led cells to die and autolysate. In contrary to the present study, Mamun *et al.* (2013) stated that the highest OD was found after 144 hours of incubation. The difference

in result comes due to, genetic constituent of their cells, cultivation condition, difference in source of the wild yeasts etc.

This study has indicated that isolate AAUTf1 did not produce hydrogen sulphide, while AAUTf5, AAUTj15 and AAUSh17 including the commercial yeast were light producer of this undesirable gas and yet other isolates produced intense dark color. Isolates exhibited much black color on Bismith Sulphate Agar (BSA) medium is designated as heavy H_2S producer (Jiranek *et al*., 1995) as noted in this study. Fellers *et al.* (1923) also reported that the highly darkened color in Lead Acetate Agar (LAA) indicates greater amount of hydrogen sulphide production. Therefore, some of the wild yeast isolates in the present study could be a potential candidate for wheat dough leavening for bread making since they showed light production of H_2S and also had better fermentation ability than commercial yeast. Furthermore, Noroul *et al*. (2013) have demonstrated that yeast strains isolated from fruits and plant parts showed better leavening performance compared to commercial strains.

Maximum leavening activity was exhibited by isolate AAU_{Tf1} (131 cm^3) followed by AAU_{Tf5} (128 cm^3) in 120 min at 30^oC. Likewise, isolates AAU_{Sh17} and AAU_{Tj15} showed better fermentative performance or leavening ability by rising the dough volume to 127 cm^3 and 125 cm^3, respectively, at 60 min of incubation temperature compared to the commercial strain (117 cm^3 at 90 minute). The results of the present study indicated that the ability of the potent yeast isolates is comparable or even better than the commercial yeast in leavening bread dough. Similarly, Maaruf *et al*. (2011) have indicated that yeast strains isolated from fruits showed higher leavening activity than that of the commercial yeast strain. Zaky and Nasr (2011) have compared the dough rising power of different brands of baker's yeasts (from Turkey, China, UK, and Egypt) sold in Egypt and all the yeast strains had maximum leavening activity after 2 hours of fermentation, but the highest leavening activity showed by the potent yeast isolates between 1 to 2 hours in the current study. This reveals that the leavening activity of indigenous elite yeast isolates showed shorter time of fermentation than that of the commercial baker's yeast (from Turkey, China, UK, and Egypt) sold in Egypt making the potent yeast isolates of this study a potential candidate to be developed into commercial bakery yeast strains after further necessary tests.

Combination of the three isolates (AAUTf1 + AAUTf5 + AAUTj15) produced the highest volume (143 cm^3) at 90 min followed by (141 cm^3) by combination of AAUTf5 + AAUTj15 at 60 min. The commercial strain that showed reasonable leavening action (117 cm^3) at 90 min was performed significantly ($p<0.05$) lower than the combined isolates ([AAUTf1 + AAUTf5 + AAUTj15], [AAUTf5 + AAUTj15], [AAUTf1 + AAUTj15 + AAUSh17], [AAUTf5 + AAUTj15 + AAUSh17], [AAUTf1 + AAUTf5 + AAUTj15 + AAUSh17] and [AAUTf5 + AAUSh17]) and the single isolate (AAUTj15 and AAUSh17). The increment in dough volume by the combination of isolates with *Saccharomyces cerevisiae* (AAUTj15 and AAUSh17) was higher compared to single inoculation. Better performance of combined wild native yeast isolates (this study) could be due to synergetic contribution of the isolates to the dough leavening action as demonstrated by several investigators (Clemente-Jimenez et al. 2005; Moreira *et al* .2008*;* Domizio *et al.* 2011; Crafack *et al.* 2013; Saerens and Swiegers, 2013; Steensels *et al*. 2014), who reported that alternative yeasts (non *Saccharomyces cerevisiae*) are not often used as monocultures (single cultures), but rather mixed with *S. cerevisiae* for quality bread baking purpose. Emphasizing the importance of uncommon yeast strains, Wedral *et al.* (2010) have demonstrated that many uncommon (non-conventional) types of yeasts used in baking industry have ability to produce unique aroma compounds but do not possess all the desirable qualities found in *S. cerevisiae* mostly since they fail to attain a desirable fermentation speed and attenuation. This in agreement to the result of this study, both single isolates of AAUTf1 (*Candida humilis*) and AAUTf5 (*Kazachistania bulderi*) have good leavening ability and aroma but took long time (about 2 hours) for dough fermentation.

In overall, it was noticed that the combinations of (AAUTf5 + AAUTj15) and (AAUTf1 + AAUTj15 + AAUSh17) indigenous yeasts, from local substrates showed highest leavening ability of bread, which contributes to the cost effective role in the production of baker's yeast; hence, increasing the varieties of yeast and decreasing its importation. Furthermore, this study may even lead to the designation of Ethiopia as the major bakery yeast producer and exporter in the horn of Africa.

6. CONCLUSIONS AND RECOMMENDATIONS

6.1. Conclusions

The results of our study demonstrated that the optimum growth conditions for yeasts are 30^{o}C temperature, 5.5 pH and 48 hours of incubation. The yeast isolate *Saccharomyces cerevisiae* exhibited good leavening activity and *Candida humilis* and *Kazchistania bulderi* have better capacity of leavening and would be considered as the most active yeasts to ferment bread dough compared to others strains including commercial yeast strain. The combination (Mixed culture) of isolates with *Saccharomyces cerevisiae* showed higher capacity of wheat dough leavening than of the single isolates (monoculture).

In general the isolation of yeasts strains from fermented foods and beverages such as *Teff* dough, *shamita* and *tej* implied that indigenous Ethiopian foods and drinks harbor potential baker yeasts which are used as dough leavening agent.

6.2 . Recommendations

Based on the findings of this study it is recommended that:

I. More research needs to be carried out on other parameters (organoleptic properties) that can enhance baker's yeast production efficiency of the strains to maximize their productivity.

II. Growth media optimization activities should be done since the main cheap raw material (molasses) commonly used at industrial scale yeast production is locally available in Ethiopia.

7. REFERENCES

Agaliya, P.J. and Jeevaratnam, K. (2013). Characterisation of the bacteriocins produced by two probiotic Lactobacillus isolates from idli batter. *Annal. Microbiol.* **63**: 1525-1535.

Akbar, A., Aamir, S., Moazzam R. K., Muhammad, A. S. and Muhammad, R. A. (2012). Yeast, its types and role in fermentation during bread making process. *Pak. J. Food Sci.* **22**:171-179.

Alcamo, I.E. (2001). Fundamentals of Microbiology. 6th ed. Jones and Barthett Publishers, London. pp. 456.

Almeida, M.J and Pais, C. (1996). Leavening ability and freeze tolerance of yeasts from traditional corn and rye bread doughs. *Appl. Env. Microbiol*. **62:** 4401–4404.

Aloys, N. and Angeline, N. (2009). Traditional fermented foods and beverages in Burundi. *Food Res. Int.* **42**: 588-594.

Altay, F., Karbancioglu-Gullet, F., Daskaya-Dikmen, C. and Heperkan, D. (2013). A review on traditional Turkish fermented non-alcoholic beverages: microbiota, fermentation process and quality characteristics. *Int. J. Food Microbiol*. **167***:* 44-56.

Arias, C.R., Burns, J.K., Friedrich, L.M., Goodrich, R.M. and Parish, M. E. (2002). Yeast species associated with orange juice: Evaluation of different identification methods. *Appl. Env. Microbiol.* **68**: 1955-1961.

Aslankoohi, E., Herrera-Malaver, B., Rezaei, M.N., Steensels, J., Courtin, C.M. and Verstrepen, K.J. (2016). Non-conventional yeast strains increase the aroma complexity of bread. *PloS One*, **11**: e0165126.

Attfield, P. V. and Bell, P. J.L (2003). Genetic Improvement of Baker's Yeasts. *Appl. Mycol. Biotechnol.* **3**: 213-240.

Bailey, J. E. and Ollis, D.F. (1977). Biochemical Engineering Fundamentals. McGraw-Hill book company, New York. pp. 411–426.

Balarable, M.M., Sani-Sambo, D.M. and Orukotan, A.A. (2017). Screening of Fermentative Potency of Yeast Isolates from Indigenous Sources for Dough Leavening. *Int. J. Microbiol. Biotechnol.* **2**: 12-17.

Balls, A. and Brown, J. (2007). Studies in yeast metabolism. *J. Biol. Chem.* **44:** 793-806.

Barnett, J. A., Payne, R. W., & Yarrow, D. (2000). Yeasts: Characteristics and Identification .3rd ed. Cambrige: Cambrige University Press. Cambridge, Cambridge University Press. pp. 18-226.

Bekatorou, A.P., Psarianos, C. and Koutinas, A.A. (2006). Production of food grade yeasts. *Food Technol. Biotechnol*. **44**:407-415.

Bekele Bahiru, Tetenike Mehari , Mogessie Ashenafi (2001). Chemical and nutritional properties of tej, an indigenous Ethiopian honey wine: variations within and between production units. *Afr. J. Food Technol*. **6**:104-108.

Bell, P.J.L., Higgins, V.J. and Attifield, P.V. (2001). Comparison of fermentative Capabilities of Commercial Baking and Wild type yeasts of different sugar media. *Lett. Microbiol*. **33**: 211-218.

Benitez, B., Gasent-Ramirez, J.M., Castrejon, F. and Codon, A.C. (1996). Development of new strains for the food industry. *Biotechnol.Prog.* **12**: 149-163.

Biolog (1993). YT Microplate: Instruction for use. Biolog, Inc.USA.

Birch, A. N., Petersen, M. A. and Hansen, A. S. (2013). The aroma profile of wheat bread crumb influenced by yeast concentration and fermentation temperature. *Food Sci. Technol.* **50**: 480–488.

Boekhout,T. and Kurtzman, C.P. (1996). Principles and methods used in Yeast classification, and over view of currently accepted yeast genera. **In**: Non conventional yeasts in bio technology. A Hand book, pp. 1- 99, (Wolf,K.ed). SpringerVelag, Berlin, Heidelberg.

Burrows, S. (1979). Baker's yeast. **In**: *The Yeasts in Rose,* pp. 349-420, (A.H. and Harrison, J.S. eds). Academic Press, Inc. London.

Campbell-Platt, G. (1994). Fermented foods: a world perspective. *Food Research Int*. **27**: 253-257.

Casaregola, S., Weiss, S. and Morel, G. (2011). New perspectives in hemiascomycetous yeast taxonomy. *Comptes rendus biologies*, **334***:*590-598.

Cavazza, A., Grando, M.S. and Zini, C. (1992). Rilevazione della flora microbica in mosti e vini. *Vignevini* **9**: 17–20.

Central Statistics Agency (2016). Annual import size of yeast and baking powder in Ethiopia. Annual report 2016.

Chavan, R. S. and Chavan, S. R. (2011). Sourdough technology- A traditional way for whole some foods: a review. *Compr. Rev. Food Sci. Food Saf.* **10**: 170-183.

Cho, I.H. and Peterson, D.G. (2010). Chemistry of bread aroma: a review. *Food Sci. Biotechnol*. **19**: 575–582.

Clara, I. M. (2015). Comparative genomics and transcriptomics in *Saccharomyce*. Doctoral Thesis. Instituto de Agroquímica y Tecnología de Alimentos (IATA-CSIC), Valencia, pp. 20-23.

Clemente-Jimenez JM, Mingorance-Cazorla L, Martinez-Rodriguez S, Heras-Vazquez FJL, Rodriguez-Vico F. (2005). Influence of sequential yeast mixtures on wine fermentation. *Int.J.Food Microbiol*. **98**:301–308.

Collado-Fernandez, M. (2003). Dough fermentation. PP. 648-655. Elsevier Science Ltd.

Crafack M, Mikkelsen MB, Saerens S, Knudsen M, Blennow A, Lowor S. (2013). Influencing cocoa flavour using Pichia kluyveri and Kluyveromyces marxianus in a defined mixed starter culture for cocoa fermentation. *Int. J. Food Microbiol*. **167**:103–116.

Dai. Z., Wang, B., Liu, Y., Shi, M., Wang, D., Zhang, X., Liu, T., Huang, L. and Zhang, X. (2014). Producing aglycons of ginsenosides in bakers' yeast. *Sci. Rep*. **4**: 3698.

Domizio P., Romani C., Lencioni L., Comitini F., Gobbi M. and Mannazzu I. (2011). Outlining a future for non Saccharomyces yeasts: Selection of putative spoilage wine strains to be used in association with Saccharomyces cerevisiae for grape juice fermentation. *Int. J. Food Microbiol.***147**:170–80.

Deak, T. and Beuchat, L.R. (1996). *Handbook of Food Spoilage Yeasts*, Crc Series in Contemporary Food Science, 2nd ed.; CRC Press: Boca Raton, FL, USA.

Dechassa Tolessa (2010). Isolation and Selection of Product Tolerant Yeasts for Efficient Production of Ethanol. MSc Thesis. Addis Ababa University, Addis Ababa, pp.35-37

De Vuyst, L. and Vancanneyt, M. (2007). Biodiversity and identification of sourdough lactic acid bacteria. *Food Microbiol*. **24**: 120–127.

Donalies, U.E., Nguyen, H.T., Stahl, U. and Nevoigt, E. (2008). Improvement of *Saccharomyces* yeast strains used in brewing, wine making and baking. Springer Berlin Heidelberg, German. pp. 67-98.

Desiye A. and Abegaz, K. (2013). Isolation, characterisation and identification of lactic acid bacteria and yeast involved in fermentation of *Teff (Eragrostis tef*) batter. *Adv. Res.Biol. Sci*. **1**: 36-44.

Díaz, C., Molina, A.M., Nähring, J. and Fischer, R. (2013). Characterization and dynamic behavior of wild yeast during spontaneous wine fermentation in steel tanks and amphorae. *BioMed. res. int.* 2013:1-13.

Edwards, W.P. (2007). The Science of Bakery Products. Royal Society of Chemistry, Cambridge.

Egli, C.M. and Henick-Kling, T. (2001). Identification of Brettanomyces/Dekkera species based on polymorphism in the rRNA internal transcribed spacer region. *Am. J. Enol. Vitic*. **52**: 241– 247.

Egwim, E., Amanabo, M., Yahaya A . and Mainuna, B. (2013). Nigerian indigenous fermented foods: processes and prospects. **In**: Mycotoxin and Food Safety in Developing Countries, Pp. 153-180, H.A. Makun (ed)., Rijeka: Intech. Academic Press, Inc.

Erbas, M., Uslu, M.K., Erbas,M.O. and Certel, M. (2006). Effects of fermentation and storage on the organic and fatty acid contents of tarhana a Turkish fermented cereal food. *J. Food Comp. Anal.* **19**: 294-301.

Esteve-Zarzoso, B., Belloch, C., Uruburu, F. and Querol, A. (1999). Identification of yeasts by RFLP analysis of the 5.8S rRNA gene and the two ribosomal internal transcribed spacers. *Int. J. Syst. Bacteriol*. **49**:329–337.

Felipe de Mendiburu. (2015). Agricolae: Statistical procedures for Agricultural Researche. R package version 1.2-3.https://CRAN.R-Project. org/package =agricolae.

Fernandez-Espinar, M.T., Esteve-Zarzoso, B., Querol, A. and Barrio, E. (2000). RFLP analysis of the ribosomal internal transcribed spacers and 5.8S rRNA gene region of the genus Saccharomyces: a fast method for species identification and the differentiation of yeasts. *Anton. Leeuw.* **78**: 87–97.

Frasse, P., Lambert, S., Levesque, C., Melcion, D., Richard-Molard, D., and Chiron, H. (1992). The influence of fermentation on volatile compounds in French bread crumb. *Food Sci.Technol.* **25**: 66–70.

Fujita, S., Yasuko, S., Shigeki, N., and Takuma, H. (2001). Multiplex PCR using internal transcribed spacer and regions for rapid detection and identification of yeast strains. *J. Clin. Microbiol.* **39**: 3617–3622.

Gallone, B., Steensels, J., Prahl, T., Soriaga, L., Saels, V., Herrera-Malaver, B., Merlevede, A., Roncoroni, M., Voordeckers, K., Miraglia, L. and Teiling, C. (2016). Domestication and divergence of *Saccharomyces cerevisiae* beer yeasts. *Cell*, ***166:***1397-1410.

Getachew Tafere (2015). A review on Traditional Fermented Beverages of Ethiopian. *J. Nat.Sci. Res.* **5**: 94-102.

Gisslen, W. (2010). *Professional Cooking, College Version*. John Wiley and Sons.

Godfray, H. C. J., Beddington, J. R., Crute, I. R., Haddad, L., Lawrence, D. and Muir, J. F. (2010). Food security: the challenge of feeding 9 billion people. *Sci.* **327**: 812-818.

Guillamo ´n JM, Sabate ´ J, Barrio E, Cano J, Querol A. (1998). Rapid identification of wine yeast species based on RFLP analysis of the ribosomal internal transcribed spacer (ITS) region. *Arch. Microbiol.* **169**: 387–392.

Hagman, A., Säll, T., Compagno, C. and Piskur, J. (2013). Yeast 'make-accumulate-consume' life strategy evolved as a multi-step process that predates the whole genome duplication. *PLoS One* 8: e68734.

Haggman, M. and Salovaara, H. (2008). Effect of fermentation rate on endogenous leavening of *Candida milleri* in sour rye dough. *Food Res. Int.* **41**: 266–273.

Hamelman, J. (2004). Bread: A Baker's Book of Techniques and Recipes. John Wiley, New York. pp. 432.

Hesham, A.E.-L., Virginia, W., Henry, O.J.O. and Julius, M. M. (2014). Phylogenetic analysis of biofuel yeasts based on 5.8S-ITS rDNA and D1/D2 26S rDNA sequences. *J. Genet. Eng. Biotechnol*. **12**: 37–43.

Hou, J., Tyo, KE., Liu, Z., Petranovic, D. and Nielsen, J. (2012). Metabolic engineering of recombinant protein secretion by *Saccharomyces cerevisiae*. *FEMS Yeast Res*. **12**: 491-510.

Jackson, C.L and Hartwell, L.H. (1990). Courtship in *S. cerevisiae*: both cell types choose mating partners by responding to the strongest pheromone signal. *Cell* **63**:1039-1051.

Jahan N, Azmuda N and Khan AR (2007). Isolation and identification of indigenous bakers' yeast. *Bangl. J.Microbiol.* **24**: 65-66

Jimoh, S. O., Ado, S. A., Ameh, J. B., Whong, C. M. Z. (2012). Characteristics and diversity of yeasts in locally fermented beverages sold in Nigeria. *World J. Eng. Pure Appl. Sci*. **2**:40-44.

Jiranek, V., Langridge, P. and Henschke, P.A. (1995). Validation of bismuth containing indicator media for predicting H_2S producing potential of Saccharomyces cerevisiae wine yeast under enological conditions. *Am. J. Enol. Vitic*. **46**: 269-273.

Johnson, E.A. and Echavarri-Erasun, C. (2011). Yeast Biotechnology. **In**: *The Yeasts, a Taxonomic Study*, pp. 652-661, (B.V., Kurtzman, P.C., Fell, J.W. and Boekhout, eds). Academic Press, Inc. Elsevier.

Joseph, R. and Bachhawat, A.K. (2014). Yeasts: Production and Commercial Uses. *Encyclo. Food Microbiol*. **3**: 823-830.

Kalui, C.M., Mathara, M. and Kutima, P.M. (2010). A review, Probiotic potential of spontaneosly fermented cereal based foods. *Afr. J. Biotechnol*. **9**: 2490-2498.

Kamel, B.S. and Stauffer, C.E. (1993). *Advances in Baking Technology*. Blackie Academic and Professional, London. pp. 179-222

Katarzyna, G. (2011). Characteristics of growth of yeasts and yeast-like fungi on chromogenic medium CHROM agar Candida (GRASO). *FEMS Yeast Res*. **57**:143-149.

Kay P. (1997). Cruising Cuisine: Fresh Food from the Galley. Int. *Marine/Ragged Mount. Press.* pp. 184.

Kelbessa Urga and Narasimha, H. V. (1998). Traditional sour dough bread (Difo Dabbo) making: I. Effects on phytic acid destruction. *Ethiop. J. Health Dev*.**12**: 167-173.

Klieger, P.C., 2004. *The Fleischmann Yeast Family*. Arcadia Publishing, Chicago, pp: 10-25.

Kohajdova, Z. (2010). Fermented cereal products, **In**: *Comprehensive Food Fermentation Biotechnology*, pp. 57-82, (A. Pandey, C.R. Soccol, E. Gnansounou, Ch. Larroche, P.S. NeeNigam, C.G. Dussap eds). Asiatech Publishers Inc., New Delhi.

Korabecna, M., Liska, V. and Fajfrlik, K. (2003). Primers ITS1, ITS2 and ITS4 detect the intraspecies variability in the internal transcribed spacers and 5.8 S rRNA gene region in clinical isolates of fungi. *Folia Microbial.* **48***:* 233-238.

Kumar, S., Stecher, G.and Tamura, k. (2016). MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molec. Biol. Evol*. **33**:1870-1874.

Kurtzman, C. P. and Fell, J.W. (2005). Biodiversity and Ecophysiology of Yeasts. **In**: *The Yeast Handbook*, pp. 11–30, (Gábor P, de la Rosa CL, eds). Berlin: Springer.

Kurtzman C.P.and Fell J.W. (2006). Yeast systematics and phylogeny-implications of molecular identification methods for studies in ecology. **In**: *The Yeast Handbook*, pp. 11-30, (C.A. Rosa, G. Peter, eds). Springer–Verlag, Germany.

Kurtzman, C.P. and Robnett, C. J. (2003). Phylogenetic relationships among yeasts of the '*Saccharomyces* complex' determined from multigene sequence analyses. *FEMS Yeast Res.* **3**: 417–432.

Kurtzman, C. P. and Robnett, C. J. (1998). Identification and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences. *Anton. Leeuw.* **73**:331–371

Kurtzman, C.P. and Fell, J.W. (1998). *The Yeast: a Taxonomic Study*. Amsterdan, Elsevier.

Lachance, M. A. and Starmer, W. T. (1998). Ecology and Yeasts. In: *The Yeasts, a Taxonomy Study*, pp. 21-30, (Kurtzman, C. P. and Fell, J. W. eds). Elsevier Science BV, Amsterdam.

Larkin, M.A., Blackshields,G., Brown,N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., Thompson, J.D., Gibson, T.J. and Higgins, D.G. (2007). Clustal W and Clustal X version 2.0. *Bioinf.* **23**: 2947-2948.

Las Heras-Vazquez, F.J., Mingorance-Cazorla, L., ClementeJimenez, J.M. and Rodriguez-Vico, F. (2003). Identification of yeast species from orange fruit and juice by RFLP and sequence analysis of 5.8S rRNA gene and the two internal transcribed spacers. *FEMS Yeast Res*. **3**: 3–9.

Leaw, S. N., Chang, H.C., Sun, H. F, Barton, R., Bouchara, J.P. and Chang, T.C. (2006). Identification of medically important yeast species by sequence analysis of the internal transcribed spacer regions. *J. Clin. Microbial*. **44**: 693–699.

Liljestrom, P.L., Tubb. R.S. and Korhola, M. (1988). 'Construction of a Stable alpha Galactosidase-producing Baker's Yeast Strain. *Appl. Environ. Microbial*. **54**: 245-249.

Linko,Yu-Yen, Javanainen, PSivi and Linko, Susan. (1997). Biotechnology of bread baking. *Trends in Food Sci. Technol*. **81**: 339-343.

Lhomme, E., Urien, C., Legrand, J., Dousset, X., Onno, B. and Sicard, D. (2016). Sourdough microbial community dynamics: an analysis during Franch organic bread-making processes. *Food Microbiol*. **53**: 41–50.

Lloyd-Evans, L.P.M. (1994). 'Biotechnology-derived Foods and the Battleground of Labelling. *Trends Food. Sci. Technol*. **5**: 363-367

Lopez, S., Prieto, M., Dijkstra, J., Dhanoa, M.S. and France, J. (2004). Statistical evaluation of mathematical models for microbial growth. *Int. J. Food Microbiol*. **96**:289-300.

Maaruf, A.G., Noroul, A.Z., Sakilah, A.M. and Mohd. K. A. (2011). Leavening ability of yeast from different local fruits in bakery product. *Sci. Malays.* **40**: 1413–1419.

Mamun-Or-rashid, A.N., Dash, B.K., Chowdhury, M.N., Waheed, M.F. and Pramanik, M.K. (2013). Exploration of potential baker's yeast from sugarcane juice: optimization and evaluation. *Pak. J. Biol. Sci.* **16**:617-623.

Mantyenen, V. H., Korhola, M., Gudundsson , H., Turkainen, H., Alferdsson, G. A., Salovaara, H. and Lindstrom, K. (1999). A Polyphasic study on the taxonomic position of industrial sour dough yeasts. *System. Appl. Microbiol*. **22**: 87-96.

Manzano, M., Cocolin, L., Longo, B. and Comi, G. (2004). PCR-DGGE differentiation of strains of *Saccharomyces sensu stricto*. *Anton. Leeuw.***85**: 23–27.

Marvin, G.E. (1931). Some of the characteristics of yeasts found in fermenting honey. *J. Agri. Res.* 43: 121-131.

Merico, A., Sulo, P., Piskur, J. and Compagno, C. (2007). Fermentative lifestyle in yeasts belonging to the *Saccharomyces* complex. *FEBS J.* **274**: 976–989.

Mike, B. and Sue, A. A. (1998). Fermented Fruits and Vegetables. A global Perspective. FAO Agricultural Services Bulletin No. 134.

Mogessie, A. (2006). The microbiology of Ethiopian foods and beverages: A review. *Ethiop. J. Biol. Sci*. **5**: 189-245

Montrocher, R., Verner, M.-C., Briolay, J., Gautier, C. and Marmisse, R. (1998). Phylogenetic analysis of the *saccharomyces cerevisiae* group based on polymorphism of rDNA spacer sequences. *Int. J. Syst. Bacteriol*. **48**: 295–303.

Mooha, L., Meron, R. and Semeneh, S. (2015). Uniqueness of Ethiopian traditional alcoholic beverage of plant origin, tella. *J. Ethn. Foods* **2**: 110 – 114.

Moreira, N., Mendes, F., de Pinho, RG., Hogg, T. and Vasconcelos, I. (2008). Heavy sulphur compounds, higher alcohols and esters production profile of *Hanseniaspora uvarum* and *Hanseniaspora guilliermondii* grown as pure and mixed cultures in grape must. *Int. J. Food Microbiol.* **124**:231–8.

Nakagawa, S. and Ouchii, K. (1994). 'Construction from a single parent of baker's yeast strians with high freeze tolerance and fermentative activity in both lean and sweet doughs. *Appl. Environ. Microbial*. **60**: 3499-3502.

Naumova, E.S., Korshunova, I.V., Jespersen, L. and Naumov, G.I. (2003). Molecular genetic identification of *Saccharomyces sensu stricto* strains from African sorghum beer. *FEMS yeast Res*. **3**: 177–184.

Navarrete-Bolanos. J.L. (2012). Improving traditional fermented beverages: how to evolve from spontaneous to directed fermentation. *Eng. Life Sci*. **12**: 410-418.

Nitayavardhana, S., Shrestha, P., Rasmussen, M.L., Lamsal, B.P., van Leeuwen, J.H. and Khanal, S.K. (2010). Ultrasound improved ethanol fermentation from cassava chips in cassava-based ethanol plants. *Bioresource technol*. **101**: 2741-2747.

Noroul, A., Ma'aruf, Z., Sahilah, A.G., Mohd, A.M., Khan, A. and Wan Aida, W.M. (2013). A new source of Saccharomyces cerevisiae as a leavening agent in bread making. *Int. Food Res. J*. **20**: 967-973.

Nout, M.J.R. (2009). Rich nutrition for the poorest cereal fermentations in Africa and Asia. *Food Microbiol*. **26**: 685-692.

Nyanzi, R. and Jooste, P.J. (2012). Cereal based functional foods, **In**: *Probiotics*, (E.C. Rigobelo ed). Tech, Rijeka, pp. 161-197.

Oda, Y., Yabuki, M., Tonomura, k. and Fukunaga, M. (1997). A phylogenetic analysis of *Saccharomyces* species by the sequence of 18S–28S rRNA spacer regions. *Yeast* **13**: 1243–1250.

Okafor, N. (2009). Fermented foods and their processing.*Fundam. Biotechnol.* **8**:5-19.

Oliveira, HC, Leao, CP. and Soares, FO. (2006). An application for control, monitoring and stimulation of baker's yeast fermentation process. Paper presented at: World Congress Computer Science, Engineering and Technology Education; Sao Paulo, Brazil. pp. 152-156.

Osinga, K.A. (1992). ‘Cenetische Verbesserung von Backereihefestammen. *Getreide, Mehl Brat.* **46**: 73-75.

Oslan, S.N., Salleh, A.B., Rahman, R.N.Z.R.A., Basri, M. and Chor, A.L.T. (2012). Locally yeasts from Malaysia: Identification, phylogenetic study and characterization. *Acta Biochimica Polonica*. **59**: 225-229.

Ozel, S., Sabanoglu, S., Çon, A.H. and Simşek, O. (2015). Diversity and stability of yeast species during the fermentation of tarhana. *Food biotechnol.* **29***:*117-129.

Park, K.S., Lee, D.K., Lee, H., Lee, Y., Jang, Y.S., Kim, Y.H., Yang, H.Y., Lee, S.I., Seol, W. and Kim, J.S. (2003). Phenotypic alteration of eukaryotic cells using randomized libraries of artificial transcription factors. *Nat. Biotechnol*. **21**: 1208–1214.

Parkouda, C., Nielsen, D.S., Azokpota, P., Ouoba, L., Amoa-Awua, W.K., Thorsen, L., Hounhouigan, J.D., Jensen, J.S., Tano-Debrah, K., Diawara, B.and Jakobsen M. (2009). The microbiology of alkaline-fermentation of indigenous seeds used as food condiments in Africa and Asia. *Crit. Rev. Microbiol*. **35**:139–156.

Pattison, T.L. and Von-Holy A. (2001). Effect of selected natural antimicrobials on baker’s yeast activity. *Lett. Microbiol*. **33**: 211.

Peu, P., Picard, S., Diara, A., Girault, R., Béline, F., Bridoux, G. and Dabert, P. (2012). Prediction of hydrogen sulphide production during anaerobic digestion of organic substrates. *Biores. Technol*. **121**: 419-424.

Plessas, S., Pherson, L., Bekatorou, A., Nigam, P. and Koutinas, A.A. (2005). Bread making using kefir grains as baker’s yeast. *Food chemis.* **93:**585-589.

Qureshi, S.K., Masud, T. and Sammi, S. (2007). Isolation and taxonomic characterization of yeast strains on the basis of maltose utilization capacity for bread making. *Int. J. Agri. Biol.* **9**: 110-113.

Rainieri, S., Kodama, Y., Kaneko, Y., Mikata, K., Nakao, Y., & Ashikari, T. (2006). Pure and mixed genetic lines of *Saccharomyces bayanus* and *Saccharomyces pastorianus* and their contribution to the lager brewing strain genome. *Appl. Env. Microbiol*. **72**: 3968–3974.

R Core Team, (2016). R: A Language and Environment for Statistical Computing. R Foundation for tatistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Randez-Gil, F., Sanz, P. and Prieto, J.A. (1999). Engineering baker’s yeast: room for improvement. *Trends biotechnol.* **17***:* 237-244.

Reinhart, P y Manville, R. (2001). The bread baker's apprentice: Mastering the art of extraordinary bread. Ten Spreed Press. New York: pp. 61.

Rhee, S.J., Lee, J.E. and Lee, Ch.H. (2011). Importance of lactic acid bacteria in Asian fermented foods. *Microbial. Cell Factories* **10**: 1-13.

Roberto, F., Silvia, G., Christian, A., Lia, R. and Antonietta, G. (2004). Comparison of cultural methods for the identification and molecular investigation of yeasts from sourdoughs for Italian sweet baked products. *FEMS Yeast Res*. **4**: 609–618.

Rose,A.H. and Harrison,J.S. (1969). The Yeasts: Biology of Yeasts.Vol.1, Academic Press, London. pp. 308-400.

Sarens, S., Swiegers, J.H. and Chr. Hansen A/S. (2012). *Enhancement of beer flavor by a combination of Pichia yeast and different hop varieties*. U.S. Patent Application 14/241,761.

Samuel, M. (2015). Probiotic Potential and Nutritional Importance of *Teff* (*Eragrostis tef (Zucc) Trotter*) *ENJERRA* - A review. *Afr. J. Food Agr. Nut. and Dev*. **15**: 9964- 9981.

Sand, C. and Rennie, R.P. (1999). Comparison of three commercial systems for the identification of germ-tube negative yeast species isolated from clinical specimens. *Diagn. Microbiol. Infect. Dis.* **33**: 223–229.

San Francisco Baking Institute Newsletter (2003). http://www.sfbi.com/pdfs/NewsF03.pdf. Retrieved, 2016-01-12.

Scanlon, M.G. and Zghal, M.C. (2001). Bread properties and crumb structure. *Food Res. Int*. **34**: 841–864.

Schieberle, P. and Grosch, W. (1991). Potent odorants of the wheat bread crump. Differences to the crust and effect of longer dough fermentation. *Z .Lebensm. Unters.Forsch*. **192**: 130–135.

Schneider, K. and Anderson, L. (2010). Yield gap and productivity potential in Ethiopian agriculture: Staple grains & pulses. EPAR Brief No. 98, Evans School Policy Analysis and Research (EPAR), University of Washington. p. 24.

Scott, S. (2011). Measurement of microbial cells by optical density. *J. Valid. Technol.* **17**:46-49.

Sefa-Dedeh, S., Cornelius, B., Amoa-Awua, W., Sakyi-Dawson, E.and Afoakwa, E.O. (2004).The microflora of fermented nixtamalized corn. *Int .J. Food Microbiol*. **96**:97-102.

Soares,F.O.,Gomes,P. and Correia,J.H. (2006). A bioreactor based on optical measurements for baker`s yeast fermentation. The 16th European Conference on Solid State Transducers, September15-18, 2002, Prague.

Spencer, J., Rawling, S., Stratford, Steels, H., Novodvorska, M., Archer, D.B. and Chandra, S. (2011). Yeast identification: reassessment of assimilation tests as sole universal identifiers. *Lett. Appl. Microbiol*. **53**: 503–508.

Steensels J., Snoek T., Meersman E., Nicolino M.P., Voordeckers K. and Verstrepen K. J. (2014). Improving industrial yeast strains: exploiting natural and artificial diversity. *FEMS Microbiol Rev*. **38**: 947–995

Steinkraus, K.H. (2009). Fermented foods. 3rd ed. PP., 45-53. Cornell University, Ithaca, NY, USA.

Suh, S., Zhang, N., Nguyen, N., Gross, S. and Blackwell, M. (2008). Lab Manual for Yeast Study. Mycolgy LAB Louisiana State University, pp 3-37.

Supanwong, K., Kazuyoshi, O. and Shinsaku, H. (1983). Environmental effects on ethanol tolerance of *Zymomonas mobilis*. *J. Microbial. Utilization Renewable Resour*. **3**: 254-260.

Tamene, M. and Dawit, A. (2014). *Evaluation of Yeast Biomass Production using Molasses and Supplements*. LAP LAMBERT Academic Publishing, Germany, pp. 3-50.

Tankeshwar, A. (2014). Lactophenol Cotton Blue (LPCB) mounts: Principle and staining protocol. 2016-05-17 Filed under Mycology. http://laboratoryinfo.com/lactophenol-cotton-blue-lpcb

Taxis C, Keller P, Kavagiou Z, Jensen LJ, Colombelli J. (2005). Spore number control and breeding in Saccharomyces cerevisiae. *J. Cell Biol*. **171**:627-640.

Teramoto, Y., Sato, R. and Ueda, S. (2005). Characteristics of fermentation yeast isolated from traditional Ethiopian honey wine, ogol. *Afr. J. Biotechnol*. **4**: 160-163.

Tetemke, M. and Mogessie, A. (1995). Microbiology of "Siljo", a traditional Ethiopian fermented legume product. *World J. Microbiol. Biotechnol*. **11**: 338- 342.

Umeta, M., West, C.E. and Fufa, H. (2005). Content of zinc, iron, calcium and their absorption inhibitors in foods commonly consumed in Ethiopia. *J. Food Compos. Anal. **18**:803–817.*

Van Hoek, W.P.M., Van Dijken, J.P and Pronk, J.T. (1998). Effect of specific growth Rate on Fermentative Capacity of Bakers' yeast. *Appl. Env. Microbiol*. **64**: 4226-4233.

Vaughan-Martini, A and Martini, A. (2011). *Saccharomyces* Meyen ex Rees. **In**: *The Yeasts: A Taxonomic Study*, pp. 733–746, (Kurtzman C.P., Fell, J.W. and Boekhout, T., eds). 5th eds. Elsevier, Amsterdam.

Vaughan-Martini, A. and Martini, A. (1998). *Saccharomyces* Meyen ex Reess. **In:** *The Yeasts: A Taxonomic Study*, pp. 358–371, (Kurztman, C.P. and Fell, J.W., eds). Elsevier, Amsterdam.

Verstrepen, K.J., Chambers, P.J. and Pretorius, I.S. (2006). The development of superior yeast strains for the food and beverage industries: challenges, opportunities and potential benefits. **In***: Yeasts in Food and Beverages*, pp. 399-444, (Querol, A. and Fleet, G. H. eds). Springer Berlin Heidelberg.

Vogel, A. and Abeba Gobezie. (1983). Ethiopian "*Tej*". **In**: *Handbook of Indigenous Fermented Foods*, pp 363-365, (Steinkraus, K.H., ed). Marcel Dekker, Inc. New York.

Walker, G. M (2009). *Yeasts*. Elsevier Inc., pp. 478-491.

Walker, K., Skelton, H., Smith, K. (2002). Cutaneous lesions showing giant yeast forms of *Blastomyces dermatitidis*. *J. Cutaneous Pathol.* **29**: 616–618.

Wedral, D. Shewfelt R. and Frank J. (2010). The challenge of Brettanomyces in wine. *LWT Food Sci. and Technol*. **43**:1474–1479.

White, T. J., Bruns T., Lee, S. and Taylor, J. (1990). Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics. **In**: PCR protocols. A guide to methods and applications. pp. 315–352, (M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White (eds), Academic Press, San Diego, Calif.

Wolfe, Kenneth H. and Shields, Denis C. (1997). Molecular evidence for an ancient duplication of the entire yeast genome. *Nat.* **387**: 708-713.

Xiao, A., Jiang, X., Ni, H., Yang, Q. and Cai, H. (2015). Study on the relationship between intracellular metabolites and astaxanthin accumulation during *Phaffia rhodozyma* fermentation. *Electron. J. Biotechnol.* **18**: 148–153.

Yigzaw, Y., L. Gorton, G. Akalu , and T. Solomon. (2001). Fermentation of *teff* (*Eragrostis tef*), grass pea (*Lathyrus sativus*) and their mixtures: Aspects of nutrition and food safety. *Lathyrus Lathyrism Newsletters* **2**:8–10.

Yarrow, D. (1998). Methods for the isolation, maintenance and identification of yeasts. **In:** *The Yeasts, A Taxonomic Study*, pp. 77–101, (C. P., Kurtzman and J. W., Fell, eds). Elsevier, Amsterdam.

Young, L. and Cauvain, S.P. (2007). Technology of Breadmaking. Berlin: Springer. p.77.

Zaky, A.S and Nasr, N.F. (2011). Easy and efficient method for measuring the rising power of baker's yeast. International Food Congress-Novel Approaches in Food Industry, pp 650-654.

Zhou, N., Schifferdecker, A. J., Gamero, A., Compagno, C., Boekhout, T., Piškur, J. and Knecht, W. (2017). *Kazachstania gamospora* and *Wickerhamomyces subpelliculosus*: Two alternative baker's yeasts in the modern bakery. *Int. J. Food Microbiol.* **250**: 45–58.

8. APPENDEXES

Appendix 1: Carbon Sources in YT MicroPlate

	1	**2**	**3**	**4**	5	**6**	**7**	**8**	9	10	11	12
A	water	Acetic Acid	Formic acid	Propionic acid	Succinic Acid	Succinic Acid Mono-Methyl Ester	L -Aspartic Acid	L-Glutamic Acid	L- Proline	D-Gluconic Acid	Dextrin	Inulin
B	D-Cellobiose	Gentiobiose	Maltose	Maltotriose	D-Melezitose	D-Melibiose	Palatinose	D-Raffinose	Stachyose	Sucrose	D-Trehalose	Turanose
C	N-AcetylD-Glucosamine	α-D-Glucose	D-Galactose	D-Psicose	L-Sorbose	Salicin	D-Mannitol	D-Sorbitol	D-Sorbitol	Xylitol	Glycerol	Tween 80
D	Water	Fumaric Acid	L-Malic Acid	Succinic Acid Mono-Methyl Ester	BromoSuccinic Acid	L-Glutamic Acid	γ-AminoButyric Acid	α-KetoGlutaric Acid	2- KetoD-Gluconic Acid	D-Gluconic Acid	Dextrin	Inulin
E	D-Cellobiose	Gentiobiose	Maltose	Maltotriose	D-Melezitose	D-Melibiose	Palatinose	D-Raffinose	Stachyose	Sucrose	D-Trehalose	Turanose
F	N-AcetylD-Glucosamine	D-Glucosamine	α-D-Glucose	D-Galactose	D-Psicose	L-Rhamnose	L-Sorbose	α-MethylD-Glucoside	β-MethylD-Glucoside	Amygdalin	Arbutin	Salicin
G	Maltitol	D-Mannitol	D-Sorbitol	Adonitol	D-Arabitol	Xylitol	i-Erythritol	Glycerol	Tween 80	L-Arabinose	D-Arabinose	D-Ribose
H	D-Xylose	Succinic Acid Mono-Methyl Ester plus D-Xylose	N-AcetylL-Glutamic Acid plus D-Xylose	Quinic Acid plus D-Xylose	D-Glucuronic Acid plus D-Xylose	Dextrin plus D-Xylose	α-D-Lactose plus D-Xylose	D-Melibiose plus D-Xylose	D-Galactose plus D-Xylose	m-Inositol plus D-Xylose	1,2-Propanediol plus D-Xylose	Acetoin plus D-Xylose

Oxidation

Assimilation

Appendix 2: Mean biomass mass of Yeasts under interaction of temperature, pH and incubation period

Temp	PH	Incu	AAU_{Tf1}	AAU_{Tf5}	AAU_{Tj15}	AAU_{Sh17}	AAU_{Ml20}	AAU_{Wt21}	Control
		24	0.555^{h-x}	0.52^{i-y}	0.51^{m-A}	0.5965^{j-A}	0.506^{D-O}	0.53^{B-P}	1.139^{A-E}
		48	0.695^{f-p}	0.6355^{f-q}	0.63^{i-w}	0.594^{j-A}	0.6185^{s-F}	0.5695^{v-K}	$1.188^{yz,B}$
		72	0.6415^{f-s}	0.603^{f-s}	0.596^{j-z}	0.64^{i-y}	0.591^{w-H}	0.848^{f-i}	1.312^{vwx}
		96	0.5345^{k-A}	0.713^{e-l}	0.5075^{m-A}	0.5635^{lAB}	0.5865^{x-H}	0.4985^{G-Q}	1.077^{C-G}
	3.5	120	0.61^{g-u}	0.493^{j-y}	0.512^{m-A}	0.466^{s-E}	0.4865^{E-P}	0.559^{w-L}	0.827^{L-O}
		24	0.616^{g-t}	0.551^{i-x}	0.6205^{i-x}	0.7475^{h-n}	0.686^{n-z}	0.686^{l-y}	1.3^{wx}
		48	0.646^{f-s}	0.598^{f-u}	0.6875^{h-u}	0.7095^{h-p}	0.7455^{i-u}	0.575^{t-K}	1.325^{u-x}
		72	0.492^{m-C}	0.6875^{e-n}	0.7325^{g-q}	0.7165^{h-n}	0.8355^{g-m}	0.778^{h-n}	1.462^{q-t}
	4	96	0.642^{f-s}	0.6335^{f-r}	0.676^{h-v}	0.5955^{j-A}	0.719^{k-x}	0.552^{yz-N}	1.236^{x-A}
		120	0.5475^{h-y}	0.525^{i-y}	0.637^{i-w}	0.5225^{n-D}	0.6835^{n-z}	0.4165^{N-T}	0.9165^{H-L}
		24	0.7745^{e-l}	0.64^{f-q}	0.83^{g-l}	0.9095^{e-h}	1.074^{f}	0.7645^{h-o}	1.218^{x-A}
		48	0.842^{d-g}	0.591^{g-v}	0.7285^{g-q}	0.7455^{h-n}	0.847^{g-l}	0.722^{h-r}	1.268^{xyz}
	4.5	72	0.899^{d-f}	0.7135^{e-l}	0.7965^{g-m}	0.824^{ghi}	0.7345^{i-v}	0.841^{f-j}	1.406^{t-w}
		96	0.805^{e-h}	0.678^{e-o}	0.775^{g-n}	0.7135^{h-o}	0.6275^{s-E}	0.7185^{i-s}	$1.168^{yz,D}$
		120	0.74^{e-m}	0.4975^{j-y}	0.715^{g-s}	0.653^{i-w}	0.524^{B-N}	0.5745^{u-K}	0.8945^{J-M}
	5	24	0.66^{f-r}	0.662^{e-p}	0.75^{g-p}	0.707^{h-p}	0.8925^{gh}	0.7105^{j-t}	1.22^{x-A}
		48	0.685^{f-q}	0.692^{e-m}	0.662^{h-v}	0.6645^{i-t}	0.719^{k-x}	0.6945^{k-w}	1.275^{xy}
		72	0.6825^{f-q}	0.7895^{e-j}	0.9085^{f-j}	0.8355^{f-i}	0.759^{h-s}	0.9335^{efg}	1.515^{p-t}
		96	0.6625^{f-r}	0.7135^{e-l}	0.847^{g-k}	0.7795^{h-l}	0.6985^{m-y}	0.789^{h-m}	1.318^{u-x}
		120	0.572^{h-w}	0.634^{f-q}	0.7825^{g-n}	0.6115^{i-z}	0.6165^{s-G}	0.585^{s-J}	0.917^{H-L}
	5.5	24	0.668^{f-r}	0.609^{f-s}	0.655^{h-v}	0.737^{h-n}	0.623^{s-F}	0.655^{m-D}	1.314^{u-x}
		48	0.6025^{g-u}	0.606^{f-s}	0.568^{k-A}	0.635^{i-y}	0.6705^{o-A}	0.5125^{F-P}	1.422^{s-v}
		72	0.7285^{e-n}	0.913^{efg}	0.6385^{h-w}	0.6855^{h-s}	0.819^{g-n}	0.5385^{A-P}	1.537^{n-r}
		96	0.7375^{e-m}	0.8835^{e-h}	0.6375^{i-w}	0.6995^{h-r}	0.777^{g-r}	0.501^{G-Q}	1.299^{wx}
25		120	0.6675^{f-r}	0.73^{e-k}	0.607^{i-z}	0.521^{n-D}	0.707^{l-y}	0.4745^{I-S}	0.9035^{J-M}
30		24	0.7975^{e-j}	0.6105^{f-s}	0.7995^{g-m}	0.779^{h-l}	0.804^{g-p}	0.83^{g-k}	1.02^{FGH}
		48	0.6415^{f-s}	0.5705^{h-w}	0.7645^{g-o}	0.736^{h-n}	0.825^{g-n}	0.734^{h-q}	1.675^{h-l}
		72	0.805^{e-h}	0.8255^{e-i}	0.716^{g-r}	0.802^{g-k}	0.8645^{g-j}	0.855^{e-h}	1.726^{f-j}
		96	0.784^{e-k}	0.6785^{e-o}	0.655^{h-v}	0.641^{i-y}	0.698^{m-y}	0.7765^{h-n}	1.723^{f-j}
	3.5	120	0.5825^{h-w}	0.4695^{j-y}	$0.54^{k-z,A}$	0.554^{l-C}	0.597^{v-H}	0.5565^{x-M}	1.627^{j-0}
		24	1.42^{c}	1.435^{bc}	1.366^{cd}	1.422^{c}	1.27^{e}	1.596^{c}	$1.162^{z,D}$
		48	1.555^{c}	1.43^{bc}	1.279^{cde}	1.052^{def}	1.09^{f}	1.682^{c}	1.683^{h-k}
		72	0.7085^{f-o}	0.615^{f-s}	0.6305^{i-w}	0.679^{i-s}	0.6065^{u-G}	0.803^{g-l}	1.711^{f-k}
	4	96	$0.5225^{l-,B}$	0.4775^{j-y}	0.439^{p-A}	0.4775^{q-E}	0.524^{B-N}	0.5895^{r-I}	1.686^{h-k}
		120	0.442^{p-D}	0.3085^{r-y}	0.308^{x-A}	0.3765^{A-E}	0.395^{L-P}	0.422^{M-T}	1.696^{g-k}
	4.5	24	1.374^{c}	1.404^{bc}	0.9525^{fgh}	0.9075^{e-h}	1.955^{b}	1.684^{c}	1.256^{xyz}
		48	1.818^{ab}	1.509^{abc}	1.562^{bc}	1.814^{b}	1.469^{d}	0.969^{ef}	1.844^{b-e}
		72	1.074^{d}	0.9785^{de}	1.167^{def}	1.016^{d-g}	1.387^{de}	0.7185^{i-s}	1.869^{bcd}
		96	0.54^{j-z}	0.601^{f-t}	0.802^{g-m}	0.4475^{t-E}	0.6505^{q-C}	0.4935^{G-Q}	1.84^{b-e}
		120	0.476^{n-C}	0.454^{k-y}	0.4345^{q-A}	0.389^{z-E}	0.572^{yz-I}	0.4475^{K-S}	1.6^{k-p}

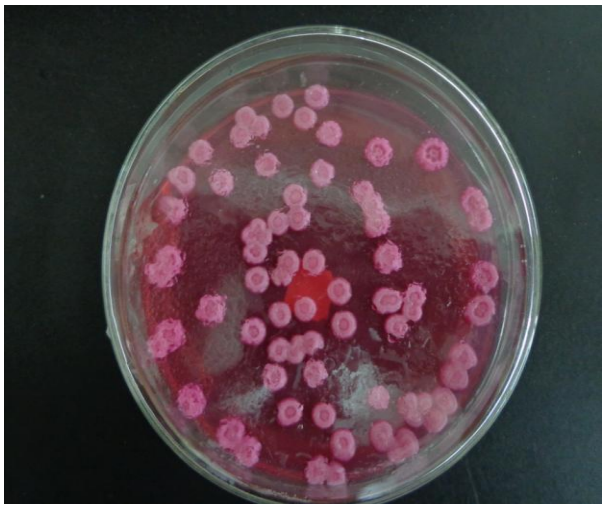
Temp	PH	Incu	AAU_{Tf1}	AAU_{Tf5}	AAU_{Tj15}	AAU_{Sh17}	AAU_{Ml20}	AAU_{Wt21}	Control
	5	24	1.564^{bc}	0.97^{de}	0.9155^{f-i}	1.132^{de}	1.284^{e}	0.986^{e}	1.261^{xyz}
		48	1.502^{c}	1.632^{ab}	1.198^{def}	1.202^{cd}	1.251^{e}	1.938^{b}	1.671^{h-l}
		72	1.62^{bc}	1.248^{cd}	0.6045^{i-z}	0.7175^{h-n}	0.875^{ghi}	1.25^{d}	2.003^{a}
		96	0.7855^{e-k}	0.713^{e-l}	0.4905^{m-A}	0.5955^{j-A}	0.7205^{k-x}	0.64^{o-F}	1.844^{b-e}
		120	0.599^{g-u}	0.6105^{f-s}	0.369^{v-A}	0.415^{y-E}	0.553^{z-J}	0.5175^{E-P}	1.761^{d-h}
	5.5	24	1.563^{bc}	1.52^{abc}	1.685^{b}	1.79^{b}	1.748^{c}	1.709^{c}	1.304^{wx}
		48	1.89^{a}	1.822^{a}	2.165^{a}	2.408^{a}	2.561^{a}	2.234^{a}	1.737^{e-j}
		72	0.9675^{de}	1.286^{cd}	1.028^{efg}	0.909^{e-h}	1.084^{f}	1.196^{d}	1.914^{abc}
		96	0.8^{e-i}	0.919^{ef}	0.832^{g-l}	0.8135^{g-j}	0.911^{g}	0.985^{e}	1.931^{ab}
		120	0.5315^{k-A}	0.5355^{i-y}	0.5765^{k-A}	0.431^{v-E}	0.617^{s-G}	0.6965^{k-v}	1.803^{d-g}
35		24	0.597^{g-v}	0.3845^{m-y}	0.645^{h-w}	0.6665^{i-t}	0.7595^{h-s}	0.759^{h-p}	1.057^{D-G}
		48	0.5345^{k-A}	0.427^{k-y}	0.6305^{i-w}	0.54^{m-C}	0.6395^{r-D}	0.6895^{l-x}	1.495^{p-t}
		72	0.5985^{g-u}	0.5215^{i-y}	0.754^{g-o}	0.8005^{g-k}	0.652^{q-C}	0.5895^{r-I}	1.756^{e-i}
		96	0.583^{h-w}	0.3485^{p-y}	0.71^{h-t}	0.6115^{i-z}	0.638^{r-D}	0.64^{o-F}	1.479^{q-t}
	3.5	120	0.487^{m-C}	0.219^{y}	0.6145^{i-k}	0.483^{p-E}	0.5385^{A-K}	0.4695^{I-S}	1.455^{rst}
	4	24	0.5095^{m-B}	0.46^{k-y}	0.5695^{k-A}	0.721^{h-n}	$0.666^{p-z,B}$	0.7805^{h-n}	0.984^{G-K}
		48	0.5545^{h-x}	0.4815^{j-y}	0.695^{h-t}	0.66^{i-u}	0.6085^{u-G}	0.625^{p-G}	1.53^{o-s}
		72	0.501^{m-C}	0.677^{e-o}	0.5545^{k-A}	0.6565^{i-v}	0.622^{s-F}	0.657^{m-C}	1.749^{e-i}
		96	0.4175^{r-D}	0.5495^{i-x}	0.4535^{o-A}	0.5305^{n-D}	0.5495^{z-J}	0.485^{H-R}	1.569^{l-q}
		120	0.326^{w-D}	0.3455^{p-y}	0.3785^{u-A}	0.388^{z-E}	0.433^{I-P}	0.4405^{K-S}	1.56^{m-r}
	4.5	24	0.5465^{i-y}	0.433^{k-y}	0.668^{h-v}	0.6625^{i-u}	0.6105^{t-G}	0.5705^{v-K}	0.984^{G-K}
		48	0.6255^{g-t}	0.48^{j-y}	0.6685^{h-v}	0.6445^{i-x}	0.635^{r-D}	0.667^{m-A}	1.735^{e-j}
		72	0.663^{f-r}	0.5055^{i-y}	0.605^{i-z}	0.591^{j-A}	0.7305^{j-w}	0.651^{n-E}	1.665^{h-m}
		96	0.5315^{k-A}	0.3675^{m-y}	0.512^{m-A}	0.4455^{t-E}	0.593^{v-H}	0.5275^{C-P}	1.672^{h-l}
		120	0.4665^{o-z}	0.3925^{l-y}	0.4205^{q-A}	0.2855^{E}	0.4865^{E-P}	0.4045^{P-U}	1.647^{i-n}
	5	24	0.5435^{i-z}	0.363^{n-y}	0.6575^{h-v}	0.549^{m-C}	0.7865^{g-q}	0.659^{m-C}	1.062^{C-G}
		48	0.5435^{i-z}	0.4115^{k-y}	0.6125^{i-x}	0.7015^{h-r}	0.603^{u-G}	0.7085^{j-u}	1.735^{e-j}
		72	0.501^{m-C}	0.464^{k-y}	0.6775^{h-v}	0.7285^{h-n}	0.6165^{s-G}	0.6175^{q-H}	1.818^{c-f}
		96	0.4435^{p-D}	0.346^{p-y}	0.5605^{k-A}	0.528^{n-D}	0.4745^{G-P}	0.4775^{I-S}	1.7^{g-k}
		120	0.377^{t-D}	0.272^{v-y}	0.494^{m-A}	0.415^{y-E}	0.4235^{J-P}	0.417^{N-T}	1.69^{h-k}
	5.5	24	0.5695^{h-x}	0.5825^{h-v}	0.716^{g-r}	0.634^{i-y}	0.754^{h-s}	0.5195^{D-P}	1.049^{EFG}
		48	0.5565^{h-x}	0.578^{h-w}	0.591^{k-z}	0.7215^{h-n}	0.6915^{n-z}	0.5692^{v-K}	1.628^{j-o}
		72	0.6665^{f-r}	0.5805^{h-v}	0.709^{h-t}	0.7585^{h-m}	0.599^{v-H}	0.785^{h-n}	1.686^{h-k}
		96	0.6385^{g-s}	0.5645^{h-w}	0.6545^{h-v}	0.7285^{h-n}	0.636^{r-D}	0.6645^{m-B}	1.472^{q-t}
		120	0.577^{h-w}	0.463^{k-y}	$0.529^{l-z,A}$	0.533^{m-D}	0.5155^{C-O}	0.4745^{I-S}	1.425^{stu}
40		24	0.2815^{A-D}	0.3085^{r-y}	0.3685^{v-A}	0.419^{x-E}	0.3935^{M-P}	0.356^{R-U}	0.999^{F-J}
		48	0.475^{p-D}	0.4515^{k-y}	0.4725^{n-A}	0.475^{r-E}	0.522^{C-N}	0.414^{O-T}	1.022^{FGH}
		72	0.3565^{u-D}	0.525^{i-y}	0.405^{r-A}	0.4585^{s-E}	0.482^{F-P}	0.3515^{R-U}	0.706^{PQ}
		96	0.287^{z-D}	0.358^{o-y}	0.298^{yzA}	0.33^{CDE}	0.382^{NOP}	0.301^{TU}	0.641^{QR}
	3.5	120	0.2145^{D}	0.2545^{wxy}	0.266^{A}	0.28^{E}	0.352^{P}	0.278^{U}	0.491^{S}
	4	24	0.418^{r-D}	0.459^{k-y}	0.508^{m-A}	0.49^{o-E}	0.3905^{NOP}	0.6155^{q-H}	1.015^{F-I}
		48	0.4505^{o-D}	0.4235^{k-y}	0.539^{k-A}	0.58^{k-A}	0.6975^{m-y}	0.45^{J-S}	1.046^{EFG}
		72	0.41^{r-D}	0.452^{k-y}	0.5895^{k-z}	0.571^{l-A}	0.7535^{h-t}	0.501^{G-Q}	0.877^{K-N}

Temp	PH	Incu	AAU_{Tf1}	AAU_{Tf5}	AAU_{Tj15}	AAU_{Sh17}	AAU_{Ml20}	AAU_{Wt21}	Control
		96	0.39^{s-D}	0.372^{m-y}	0.498^{m-A}	0.535^{m-D}	$0.622^{s-z,F}$	0.469^{l-S}	0.8045^{M-P}
		120	0.245^{CD}	0.3235^{q-y}	0.4015^{s-A}	0.4495^{t-E}	0.4985^{D-O}	0.4945^{G-Q}	0.541^{RS}
	4.5	24	0.369^{t-D}	0.277^{t-y}	0.557^{k-A}	0.7045^{h-q}	0.5375^{A-L}	0.681^{l-z}	1.065^{C-G}
		48	0.4415^{p-D}	0.342^{p-y}	0.615^{i-x}	0.624^{i-y}	0.811^{g-o}	$0.548^{z,O}$	1.057^{D-G}
		72	0.432^{q-D}	0.3275^{q-y}	0.592^{k-z}	0.717^{h-n}	0.596^{v-H}	0.5445^{A-O}	0.7455^{OPQ}
		96	0.378^{t-D}	0.273^{u-y}	0.5685^{k-A}	0.5575^{lAB}	0.4885^{E-P}	0.514^{F-P}	0.7705^{NOP}
		120	$0.2895^{yz,D}$	0.234^{y}	0.3985^{t-A}	0.4365^{u-E}	0.3925^{M-P}	0.428^{L-T}	0.635^{QR}
	5	24	$0.34^{v-z,D}$	0.294^{s-y}	0.611^{i-y}	0.725^{h-n}	0.722^{j-x}	0.6585^{m-C}	1.068^{C-G}
		48	0.4665^{o-D}	0.318^{q-y}	0.5595^{k-A}	0.6285^{i-y}	0.854^{g-k}	0.737^{h-q}	1.068^{C-G}
		72	0.4825^{m-C}	0.3615^{o-y}	0.6175^{i-x}	0.639^{i-y}	0.5905^{w-H}	0.671^{l-A}	0.888^{KLM}
		96	0.372^{t-D}	0.3065^{s-y}	0.5345^{k-A}	0.544^{m-C}	0.4595^{G-P}	0.6215^{q-G}	0.707^{PQ}
		120	0.271^{BCD}	0.275^{u-y}	0.469^{n-A}	0.468^{s-E}	0.3995^{K-P}	0.4985^{G-Q}	0.505^{S}
	5.5	24	0.3925^{s-D}	0.384^{m-y}	0.4015^{s-A}	0.428^{w-E}	0.4835^{F-P}	0.424^{L-T}	1.106^{B-F}
		48	0.465^{o-D}	0.4625^{k-y}	0.4605^{o-A}	0.5225^{n-D}	0.534^{A-M}	0.5185^{E-P}	1.106^{B-F}
		72	0.519^{l-B}	0.356^{o-y}	0.4755^{n-A}	0.443^{t-E}	0.4745^{G-P}	0.4255^{L-T}	0.9055^{I-M}
		96	0.396^{s-D}	0.3495^{p-y}	0.34^{w-A}	0.3365^{B-E}	0.4225^{J-P}	0.372^{Q-U}	0.7045^{PQ}
		120	0.3125^{x-D}	0.393^{l-y}	$0.296^{z,A}$	0.309^{DE}	0.3765^{OP}	0.343^{STU}	0.5425^{RS}

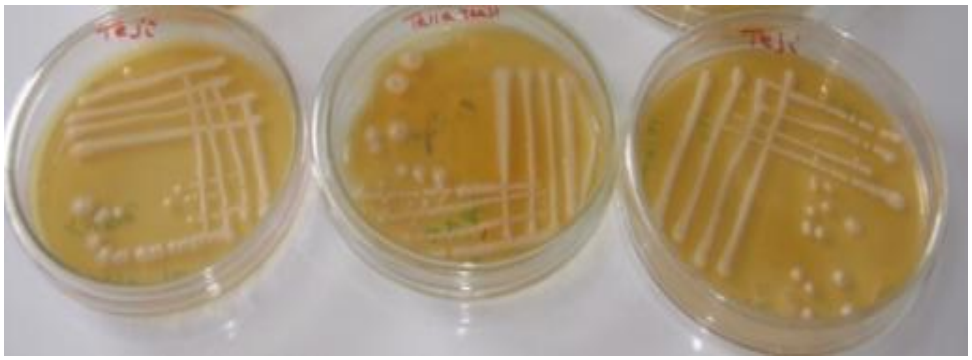
Means with the same letter are not significantly different at $p<0.05$.

Round 1 small letter a-z; round2 capital letter A

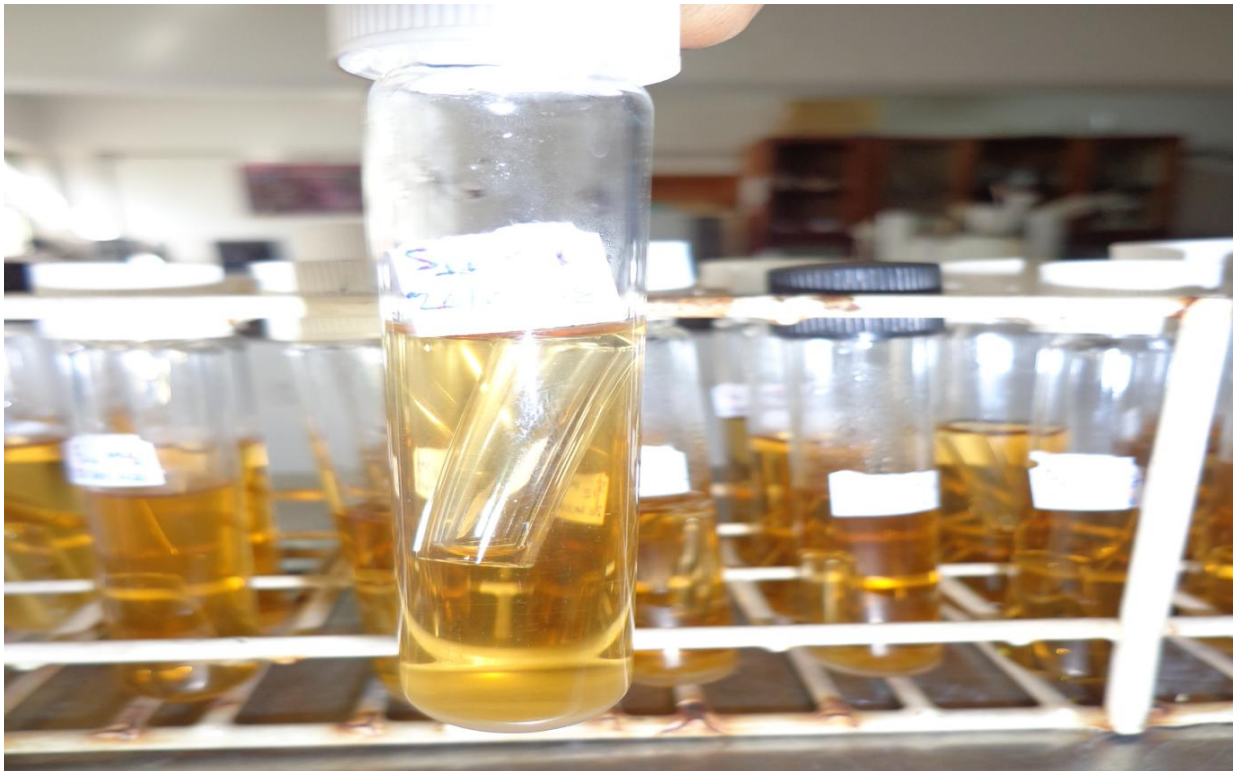
Appendix 3: Isolation of yeasts using RBCA (selective) medium



Appendix 4: Purification of yeasts using YEPDA medium



Appendix 5: Durham tube carbohydrate fermentation set up



Appendix 6: Dough fermentation by selected isolates at room temperature

Appendix 7: Dough fermentation by selected isolates at 30^{o}C

DECLARATION

I, the undersigned, declare that this thesis is my original work and has not been presented for any other awards at this or other university and all the sources of material used for this thesis have been dually acknowledged.

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Date of Submission: _____/_____/_____

This MSc thesis has been submitted for examination with our approval as University Advisors.

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