TECHNOLOGICAL CHARACTERISATION OF YEAST STRAINS ISOLATED FROM THE TRADITIONAL FOODS.

Submitted by

Rimjhim Agarwal (131551)

Shivani Agarwal (131569)

Under the supervision of

Dr.GunjanGoel

Associate Professor

May 2017

Submitted in fulfilment of the degree of
4 Year Degree Programme B.Tech

DEPARTMENT OF BIOTECHNOLOGY AND BIOINFORMATICS
JAYPEE UNIVERSITY OF INFORMATION TECHNOLOGY
WAKNAGHAT (H.P)

DEPARTMENT OF BIOTECHNOLOGY AND BIOINFORMATICS
JAYPEE UNIVERSITY OF INFORMATION TECHNOLOGY,
WAKNAGHAT (H.P)

CERTIFICATE

This is to certify that Miss Rimjhim Agarwal and Miss Shivani Agarwal have carried out the undergraduate dissertation project work on "**TECHNOLOGICAL CHARACTERISATION OF YEAST STRAINS ISOLATED FROM THE TRADITIONAL FOODS**" under my supervision form July 2016 to May 2017. During the project work they both had learnt most of the techniques used during the work.

The work presented in this project report is original and has not been submitted anywhere else for any other degree.

Dr.GunjanGoel
Associate Professor
Department of Biotechnology and Bioinformatics,
Jaypee University of Information Technology,
Waknaghat, Dist. Solan, H.P

ACKNOWLEDGEMENT

We express our gratitude to the almighty for blessing in successive completion of this dissertation. We would like to acknowledge a few of the people who made this project possible.

We take this golden opportunity to express our gratitude and sincere thanks to our respected research guide Dr.**GunjanGoel**, Associate Professor, Department of Biotechnology, Jaypee University of Information Technology, Waknaghat for his constant and valuable guidance, encouragement and support which boosted our confidence.

We would like to give special thanks to Dr. R.S Chauhan, the dean and head, Department of Biotechnology and Bioinformatics for providing facilities which enabled us to complete this project work successfully.

We wish to express our deep gratitude to **Mr SampanAttri**,Phd scholar, who extended valuable encouragement, suggestions and advises throughout the project work. There constant assistance and kindness in editing this thesis manuscript is highly appreciated.

We would like to thank our family who taught us the value of hard work by their experience.

Date:

Rimjhim Agarwal (131551)

Shivani Agarwal (131569)

DECLARATION

I do here by declare that the dissertation entitled **"TECHNOLOGICAL CHARACTERISATION OF YEAST STRAINS ISOLATED FROM THE TRADITIONAL FOODS"** submitted towards fulfilment for the award ofdegree of **Bachelor of Technology in Biotechnology** of Jaypee University of Information Technology is based on the results of studies carried out under the guidance and supervision of **Dr.GunjanGoel**. This dissertation or no part of this has been submitted elsewhere for the award of any degree or diploma.

Shiavni Agarwal
131569

Rimjhim Agarwal
131569

Table of Contents

List of Figure:

List of Table

Chapter-1
INTRODUCTION

INTRODUCTON:

The traditional fermented food and beverages contribute to a major portion of staple diet of people belonging to rural/tribal/himalayan regions of Lahaul and Spiti, Kinnaur, Kullu, Shimla, Kangra districts of Himachal Pradesh. These traditional foods consists of natural starter cultures locally known as *khameer/Phab/lang/ Malera*. These are generally rich in yeast and bacterial micro flora and are till now restricted to household levels only. These foods are part of the diet of tribal people since decades, also fermented foods are treasured as major dietary constituents in numerous developing countries because of their keeping quality under ambient conditions - thereby contributing to food security - and because they add value, enhance nutritional quality and digestibility, improve food safety, and are traditionally acceptable and accessible (Holzapfel, 2002, Rolle and Satin, 2002). They have beneficial activities such as theyboost up the nutritional value of the food by producing essential amino acids, SCFAs, enzymes, and increase bioavailability of minerals, hence indicating that these do not have any harmful effects on human system. Fermented foods had been used since pre-historic era and is one of the popular method for food preservation throughout the world.

The significance of fermentation in present day is characterized by the broad spectrum foods which are marketed in both industrialized as well as developing countries, by not only considering the aids of food preservation and its safety but also for their high sensory attributes.

Fermentation is a low-input enterprise and provides individuals with limited purchasing power, access to safe, inexpensive and nutritious foods. Preservation and safeguarding of foods and beverages remain the principal objectives of fermentation, with wholesomeness, acceptability and overall quality, having become increasingly valued features to consumers, especially in rural areas where old traditions and cultural particularities in food fermentations are generally well maintained. The tradition of fermented beverages is long embedded in many cultures, and despite traditional production technologies remaining, there is potential for extension services to introduce some improved methods, particularly those for hygiene and safety.

Increasing knowledge on the benefits of fermented foods and customers gaining interest in adapting traditional practices provides a greater market for such novel strains which are restricted in these regions.

Rationale behind this study:

- Subsequent investigation and future potentials for applications of these strains.
- Information on the characteristics of the micro-organisms isolated from the ethnic fermented foods significantly enriches the database of the microbial diversity from food ecosystem in the Himalayas.
- Some strains can be exploited for the production of enzymes, bioactive compounds and other industrial uses.
- Therapeutic values found in the traditional starter in the Himalayas typically, additional medicines or supplementary drugs are usually not required.

Based on above rationale, the aim of present study was to characterize the yeast strains isolated from the traditional foods from the upper Himachal region and compare it with the commercial yeast strain used for the production of wine in terms of Alcohol tolerance, sugar tolerance, and pH tolerance to check which one is better for commercial purpose with following objectives:

- Characterization and comparison of yeast strain isolated from traditional beverages
- Preparation and analysis of wine prepared from the isolated yeast strains

Chapter-2

REVIEW OF LITERATURE

Review of Literature:

The term 'Fermentation' is derived from the Latin verb, *fevere*, to boil. Fermentation technology is one of the oldest food technologies that have been used for several thousand years as an effective and low cost means for preserving foods and beverages. Food fermentation is of prime importance in the developing countries where the limitations of resources encourages the use of locally available fermented food products for additional nutrition. These fermented products are more common among people belonging to rural areas, without much awareness about the microflora associated with these products. This microflora involves a combination of bacteria, yeast, fungi which have been reported by several workers from various fermented foods[5].

In India around INR 133 billion of fruits are wasted annually. In order to avoid such wastage of fruits, local people of India extract fruit juice and ferment them in order to preserve its nutritional value. Also, its antioxidant properties of the fermented product enhances due to metabolism of the microbe used in fermentation.

2.1 *Phab* preparation

Phab is the local inoculum found in trans-Himalayan area of northern India. It is essentially said to be a dried out yeast plan. Be that as it may, couple of bacterial species have additionally been accounted for *Phab*arrangement is a temperature dependent microbial process in which *S.cerevisae* and *Bacillus* species have been accounted for [2].

The great months: mid June to early October

Temperature: 28°C to 38°C

Relative humidity: 44% to 51.5%.

A *Phab* has a restorative significance in customary medication system. An amchi nearby practioner utilize *Phab*s in hydrotherapy alongside different plants to treat joint pain and joint torment. *Phab* planning is temperature dependent process. It is utilized for the arrangement of *chhang, lugri, aara, daru, angoori, uthi and behmi.*

Fig. 1: The traditional inoculum, *'Phab'*

2.1.1**Artemisia sp.:** *Artemisia* is a yellow colored wild blooms having strong aromaticity. It is developed in groups on the edges of the water system waterways or on the patio fields. *Artemisia* species have likewise been accounted for to have an inhibitory impact on the development of some nourishment borne bacterial species (Erelet al.2012). These are utilized for incubating amid the aging stride if there should arise an occurrence of *Phab* readiness[3].

Fig.2: *Artemisia sp.* locally called as *burnak*

2.1.2 **Preparation of *Phab:*** Preparation of *Phab* includes the utilization of husked grain, grains which are actually matured lastly casted into the type of tablets. These are then sundried later so they can be put away for long term without freezing. The utilization of *Phab* is well-predominant in Himachal Pradesh for the fermentation of mixed drinks which is served during important events[3].

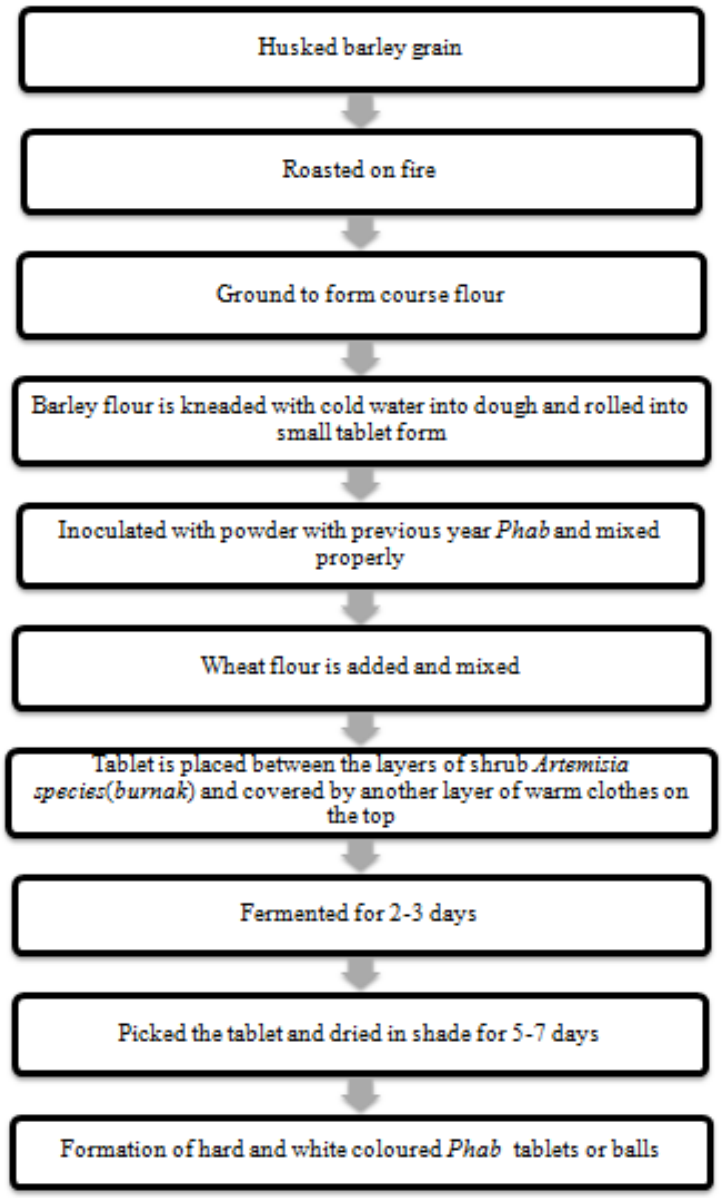

Fig. 3: Flowchart showing the preparation of *Phab* (KunzesAngmo and T C Balla,2014)

2.2 ***Malera***

Malera is fermented wheat flour mixture which is widely utilized for preparing *bhaturu*. Be that as it may, it is additionally utilized for the preparing *daru* in couple of inner districts of Himachal Pradesh. Preparing of this includes fermenting it naturally[3].

2.3 **Fermented foods and beverages of Himachal Pradesh**

Conventional fermented food and beverages are extremely well known in the tribal and country regions of Himachal Pradesh. Various fermented foods and beverages were recognized and the conventional fermentation procedures were examined. A portion of the prevalent aged beverages and food were analysed for their microbiological attributes. The fermented items that are one of a kind to the tribal and country belts of Himachal are *Bhaturu, Siddu, Chilra, Manna, Marchu, Bagpinni, Seera, Dosha, Sepubari, Sura, Chhang, Lugri, Daru, Angoori and Behmi*. Other than using this as source of nutrition, these fermented foods e.g. *Bhaturu*, constitute staple food in large part of provincial zones of Kullu, Kangra, Mandi and Lahaul and Spiti regions of the state while others are consumed during festivals, relational unions and special events. Customary starter cultures like "*Phab*" (dried out yeast), "*Treh"* (already fermented wheat flour slurry) and "*Malera*" (beforehand fermented wheat flour batter) are the inocula utilized as a part of get ready aged items[4].

Table1: Some of the fermented beverages of Himachal Pradesh (Thakur et al., 2003)

Fermented beverages	**Substrate**	**Area**
Sura	Millet flour	Kullu
Chhang/Lugri	Rice/Barley	Lahaul&Spiti, Kullu and Kinnaur
Daru/Chakti	Jaggery	Shimla, Kullu
Angoori/Kinnauri	Grapes	Kinnaur
Chulli	Wild apricot	Kinnaur
Arak/Ara	Apple, Chulli, Barley, Pear	Kinnaur
Behmi		Kinnaur

2.4 **Fermented foods and beverages from *Phab* and *Malera***

Generally these foods are cereal based (wheat/grain/buckwheat) however some legumes (dark gram) and milk based fermented foods andbeverages are commonly used. Products like *Bhaturu, Siddu, Chilra, Marchu, Manna, Dosha, Pinni/Bagpinni, Seera,*etc. are unique to Himachal Pradesh. In the tribal areas of Lahaul and Spiti and Kinnaur, substantial assortment of fermented food is prepared either by day to day, at special events or for consumption during trip. Conventional starter cultures like "*Malera*" and "*Treh*" are used as inocula in making these fermented products.However, the regular fermentation (without inoculating inoculum, as microorganisms present in the crude materials do fermentation) is utilized as a part of the creation of *Seera, Sepubari and Borhe,* and so on[1].

2.5 **Yeast:**

Yeasts are single celled, eukaryotic microorganisms which are classified as members of the kingdom fungus. Yeasts are unicellular organisms which evolved from multicellular ancestors with some species having the ability to develop multicellular characteristics by forming strings of connected budding cells known as pseudo-hyphae or false hyphae[6]. Yeast were originated 100 of millions ago and 1,500 species of it have been identified till date and they constitute 1% of all described fungus species.

Size of yeast varies from species to species, environment in which Yeast sizes vary greatly, typically yeast is 3–4 μm in diameter, although some yeasts have the tendency to grow to 40 μm in size[7]. Mostly yeasts reproduce by asexually means i.e by mitosis, also they can reproduce by the means of asymmetric division process which is known as budding[12].

The physiological properties of yeast which could be exploited in the field of biotechnology and its emergence. Fermentation of sugars by yeast is one of the oldest and biggest application of this technology[8]. Many types of yeasts can be used for production of many types of foods and beverages which are consumed worldwide For example: baker's yeast that is used for making of bread, brewer's yeast in making of beer, and yeast in fermenting wine and also for xylitol production[9].

Beverages that contain ethanol (C_2H_5OH) are known as Alcohol beverages[10]. This ethanol is produces due to the conversion of sugar into alcohol in the anaerobic condition in presence of low-oxygen by certain species of yeasts. Beverages such as wine, whiskey, beer, and distilled spirits all are produced by the use yeast at some stage of their making[11].

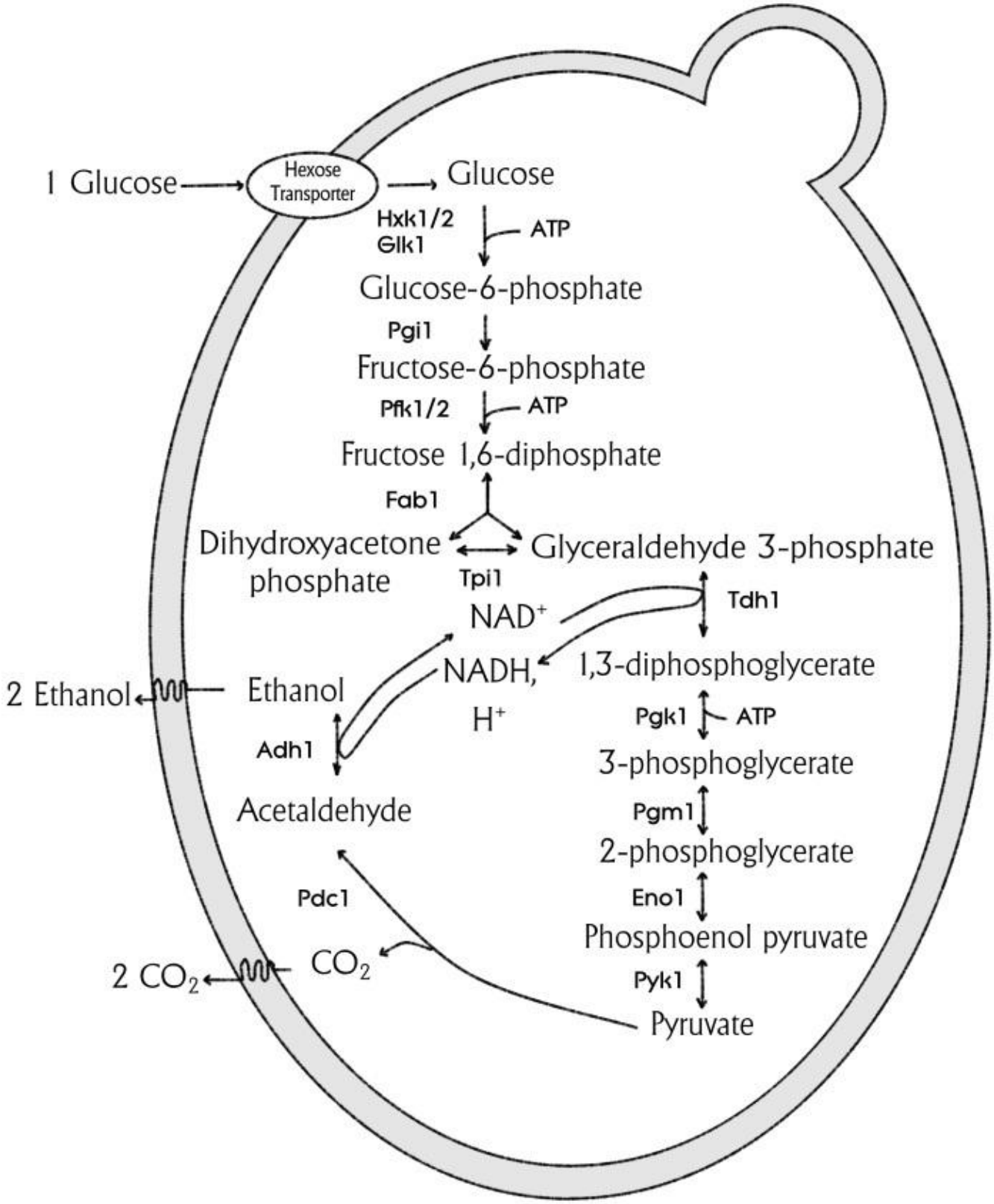

Figure 4: A scheme of ethanol production pathway by yeast. (Source: http://www.intechopen.com/source/html/41685/media/image1.jpeg

2.6 **Wine Making:**

Wine is fermented product of fruit juice which is commercially fermented with commercial yeast strain known as *Saccharomyces cerevisaie*. It convert sugar into ethanol. Wine contains approximately 12% to 15% alcohol concentration and contains sensory aroma in it.

Wine productions involve many steps which are as follows:

Grapes selection: grapes which are undamaged and mature are selected for fermentation process.

A. **Crushing of Grapes**: crushing can be done with the help of crushers as well as manually, the pulp of grape is taken out and the skin of the grapes are removed with the help of stainless steel sieve and remaining pulp and juice is taken out of it.
B. **Clarification:** juice of grape is then sieved 2-3 times and centrifugation is done in order to clarify it.
C. **Fermentation**: clarified grape juice is then transferred to the fermentation unit where starter culture is added and juice is left undisturbed for weeks in order to ferment it.
D. **Ageing**: Aging of wine is done where flavor of the wine enhances.
E. **Maturation and Bottling**: It takes 6-8 months for maturation of the wine once it's matured enough it is pasteurized to remove any other contamination.
F. **Packaging and storage**: traditionally wine is packed in glass bottles covered with cork. Bottles are then stored in dark and during its storage it is made sure the wine don't get spoiled by any other microorganisms[14].

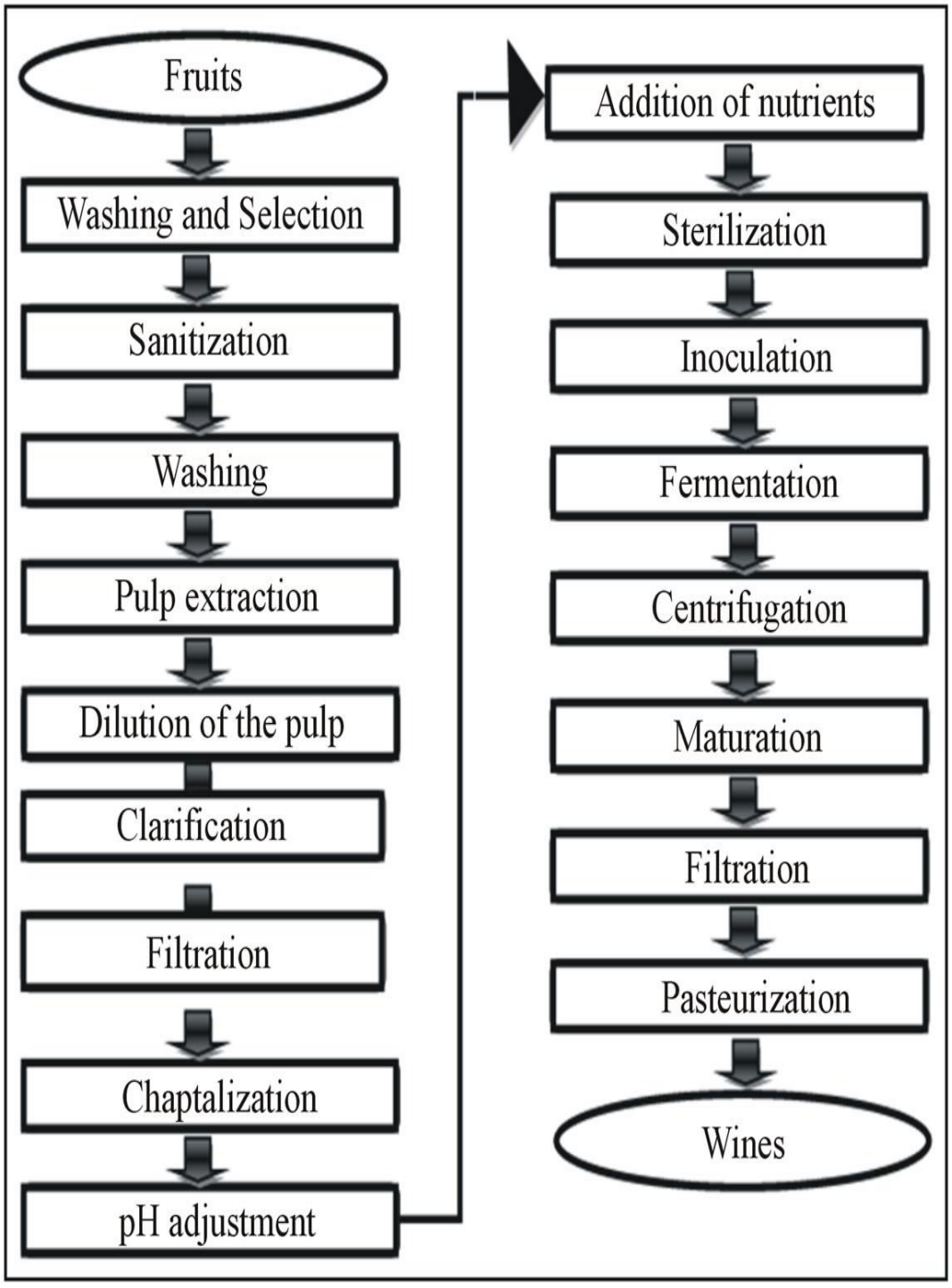

Figure 5: Flowchart showing steps for Preparation of wine

Chapter-3
Materials and Methods

Material and Method:

3.1 Maintenance of yeast cultures:

Yeast cultures from *Malera, Phab,* Lahn were already isolated from the traditional foods of Himachal Region in our lab and the commercial brewing strain of yeast(MTCC...) was taken from the microbial stock cultures in our department. The yeast cultures were maintained on Malt extract medium and preserved as glycerol stock .

For our investigation, a 48 hours revived culture of all the strains was streaked on PDA plates and were kept at 37 degrees for 48 hours. Plates were examined for morphological characteristics like colour, shape of colony, variety of colonies on the plate etc. This was done to check the purity of glycerol stocks which will be used for further characterisation of the strains.

3.2 Oenological Characterization of Yeast strains:

3.2.1 Alcohol Tolerance Test:

A 100 ml of Malt Extract Broth was taken in 250 ml capacity flask each and 1% inoculum of each of the strain was added to each flask with addition of ethanol such that concentration of media becomes 0%, 5%, 10%, 15% and 20% for both of the yeast strain.

Then all the flasks were left for incubation at 37º for 48 hours. Samples were collected in 2ml tubes after every 6 hours and readings were taken of the sample collected with the help of spectrophotometer at 600 nm wavelength after 48 hours.

3.2.2 pH tolerance Test:

Malt Extract Broth (50mL}was taken in 250 ml capacity flasks each and 1% inoculum was added to each flask and pH was set to 2.0, 3.0, 4.0 and 5.0 for all five yeast strains.

Then all the flasks were kept in incubators with 37ºC temperature for 48 hours for incubation and samples were collected in 2ml tubes after every 6 hours and readings were taken of the sample collected with the help of spectrophotometer at 600 nm wavelength after 48 hours.

3.2.3 **Sugar tolerance test:**

Malt Extract Broth (50mL) was taken in 250 ml capacity flasks each and 1% inoculum was added to each flask such that concentration of the media becomes 0%, 5%, 10%, 5%, 20% for all five yeast strains.

Then all the flasks were kept in incubator at 37ºC temperature for 48 hours for incubation and samples were collected in 2ml tubes after every 6 hours and readings were taken of the sample collected with the help of spectrophotometer at 600 nm wavelength after 48 hours.

3.3 **DNA ISOLATION AND PCR AMPLIFICATION:**

A 2ml aliquot of overnight grown yeast culture in Malt Extract media was taken in 2ml eppendorfs. The samples were then centrifuged for 2 minutes at 10,000rpm and supernatant was discarded. 1ml of the extraction buffer was added in the tube having the pellet. The tubes were vortexed for 30 seconds. The tubes were then put into the water bath having 70º C temperature for 30 minutes with constant vortexing. The 2mL tube was then centrifuged at 10000rpm for 2 minutes. The supernatant was taken in another tubes tube and pellet was discarded. Then 1ml of phenol:chloroform in 1:1 ratio was added in the tubes containing supernatant. The sample was then vortexed and centrifuged at 10000 rpm for 2 minutes. After centrifugation 3 layer supernatant was formed from which top most layer was taken out and transferred to another 2 ml tubes. 1 ml of chilled isopropanol was added to the supernatant and tubes was inverted to and fro for mixing. Tubes were incubated for 30 minutes at -80 degree celsius. After incubation all the tubes were centrifuged to 10000 rpm for 10 minutes. Supernatant was discarded and pellet was washed with 500 ul chilled ethanol. All the eppendorfs were then centrifuged to 10000 rpm for 2 minutes. The obtained pellet was dried and 50ul of autoclaved water with 3 ul RNase were added to the pellet and the DNA sample was stored.

Next day, Nano drop of the sample was done. After desired readings from Nano drop, PCR reaction was setup without GC content and PCR was run. After PCR amplification Agarose gel (1.2%) was run with the amplified samples to check for the amplification.

Table 2: Composition of lysis buffer

Components	Quantity for a reaction(µL)
TrisHCl	200
EDTA	100
NaCl	100
Water	400
SDS	100

Table 3:Composition of PCR master mix

Composition of Master mix	1X(µL)
Distilled water	11.05
Buffer	1.5
dNTPs	0.3
Primer(F)	0.5
Primer(R)	0.5
Polymerase(Taq or Pfu)	0.15
DNA template	1.0

A desired PCR amplified product of 800 bp was excised from gel, eluted in 3ul of nuclease free water and sent for sequencing.

3.5 **WINE TESTS:**

3.5.1 **Preparation of wine**:

A. Preparation of juice:

Ripened grapes (3.5 Kg) were washed under water. Fresh juice was extracted from ripened grapes by mashing them. Juice (2L) was filtered using muslin cloth multiple times to reduce the amount of raw grape content.

B. Setting up the required brix:

Sugar content was measured before the activation of yeast cells which was to be 18º brix. Sucrose (35g\L) was added to juice to bring the juice at 22^0 brix each.

C. Preparation of yeast cells:

Yeast cells were centrifuged to pellet them down from the reviving media. The pellet was washed with 0.85% saline solution. OD of the cells was taken at 600nm with saline solution being the blank.

D. Activation of cells:

Prepared cells were then inoculated in 100ml of juice at 37degrees without disturbance for 2 hours.

E. Fermentation step:

Activated cells along with juice were then transferred to the remaining juice. The juices were then left for fermentation for 6days. Samples were collected each day from the fermentation unit for further analysis of prepared wine.

F. Filtration and pasteurization of wine:

Fermented juice was left at 4degrees for a month for proper sedimentation and ageing of wine. Clarified juice was centrifuged at 10,000rpm for 10 min at room temperature to settle the remaining grape pulp. The supernatant was transferred to fresh bottle. The juice was then pasteurized in water bath at 70degrees for 30 min and was then immediately stored at 4 degrees.

3.6 **Analysis of prepared wine:**

3.6.1. ABTS Assay:

The scavenging activity was estimated according to procedure (Pellegrino et. al., 1993). ABTS (7 mM) in water was prepared by mixing a stock solution with potassium per sulphate (2.45 mM) in an equal quantity and left to stand for 12-16 hours at room temperature in dark until reaching a stable oxidative state. The ABTS solution was diluted with 80% ethanol to an absorbance of 0.80 ± 0.05 at 734n. 100 µl of sample was mixed with 2.9 ml of ABTS solution and the mixture was allowed to stand at room temperature for 30 min dark condition. The absorbance was determined at 734nm.

Scavenging effect (%) = {1 – (Absorbance sample / Absorbance control)} X 100

3.6.2 Total soluble solute test:

A 1ml each of sample was taken on refractometer and the readings were noted for all the samples collected from 4 wines prepared with different cultures for each day.

Chapter-4
Result and Discussions

Results:

4.1 Yeast cultures

All the yeast cultures were obtained as pure cultures on Malt extract agar as indicated by uniform, well isolated colonies which were white in color. The microscopic observation indicated the **typical yeast morphology of the cells with few cells in the budding form.**

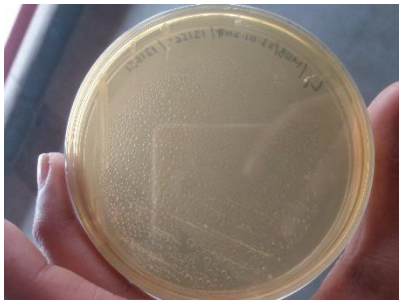

Figure 6: Malt extract agar plate of Lahn

Figure 7: Malt Extract agar plate of Phab

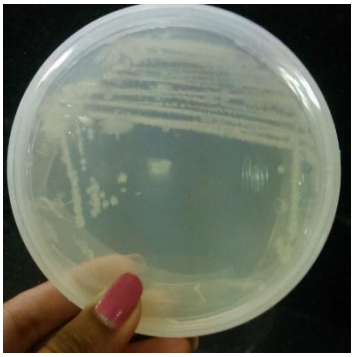

Figure 8: Malt extract agar plate of Malera

4.2 **Oenological characteristics:**

5.2.1 Alcohol Tolerance:

All the selected yeast strains were subjected to ethanol tolerance test at a level of 0-20% in Malt extract broth, where an aliquot of 2 ml was taken at different time intervals. The effect of ethanol tolerance was determined by optical density of the growth at 600nm.

It can be inferred from the graphs that ethanol tolerance for the strains is as follows:

Range taken 0-20% with interval of 5%

- Yeast Strain from *Phab*- 5%
- Yeast Strain from *Lahn*- 5%
- Yeast Strain from *Malera*- 6%
- *Saccharomyces cervisiae*-6%

As tolerance for *Malera* and *Saccharomyces cervisiae* is same to ethanol, therefore we will use fermentation time as the distinguishing factor for the strains. It is inferred that though *Malera*' s growth pattern is similar to that of commercial yeast strain but it has a lesser doubling time (*Malera*-30 hours, commercial strain- 42 hours).

This can benefit in the following manner:

- Decreasing cost and energy consumption.
- Allows more ethanol production per batch
- Conservation of traditional strain
- Can be used for industrial purposes hence, rendering benefits to local people on traditional knowledge and geographical indications.

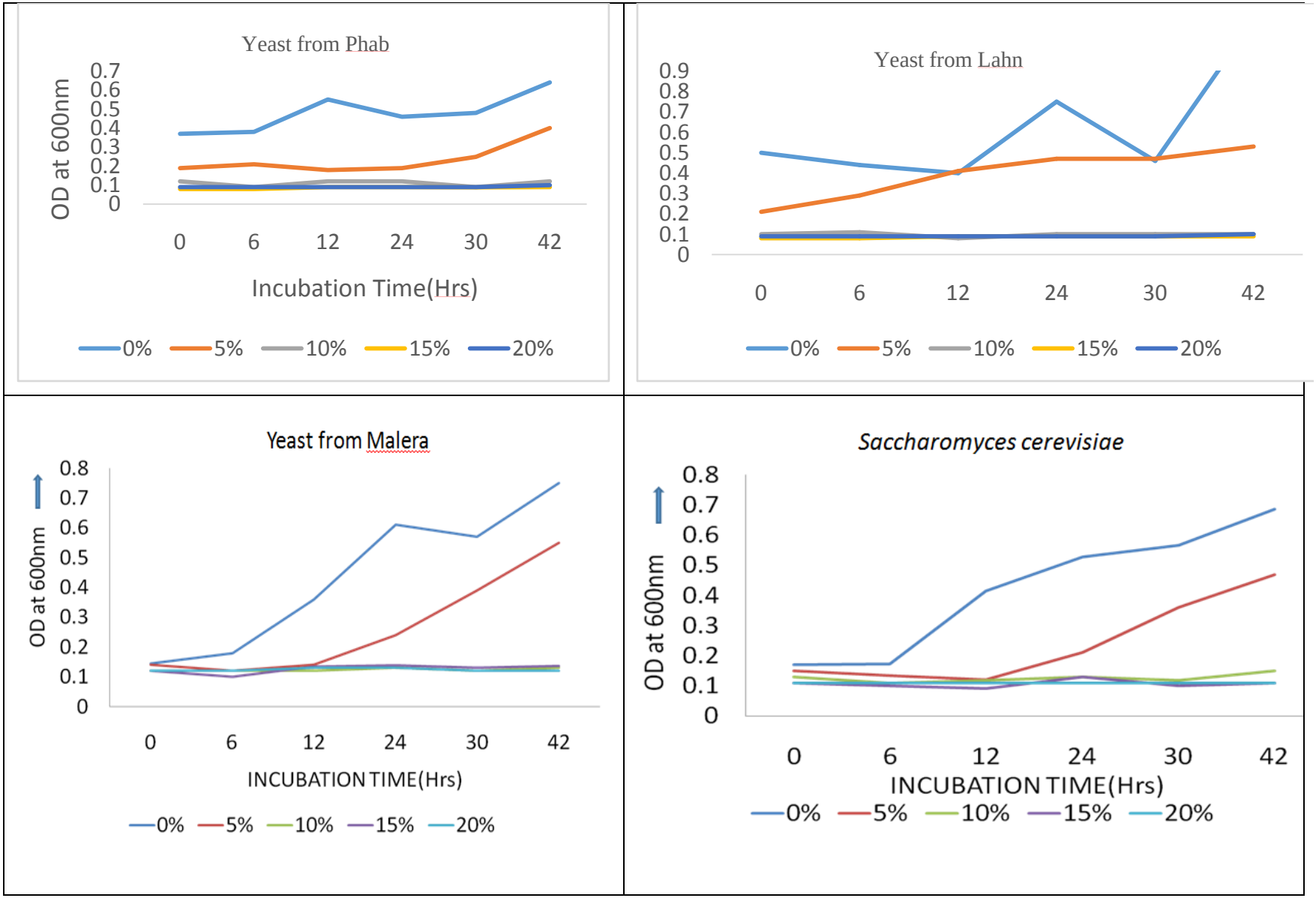

Figure 9: Graphs showing Alcohol Tolerance of different cultures.

5.2.2 Sugar Tolerance:

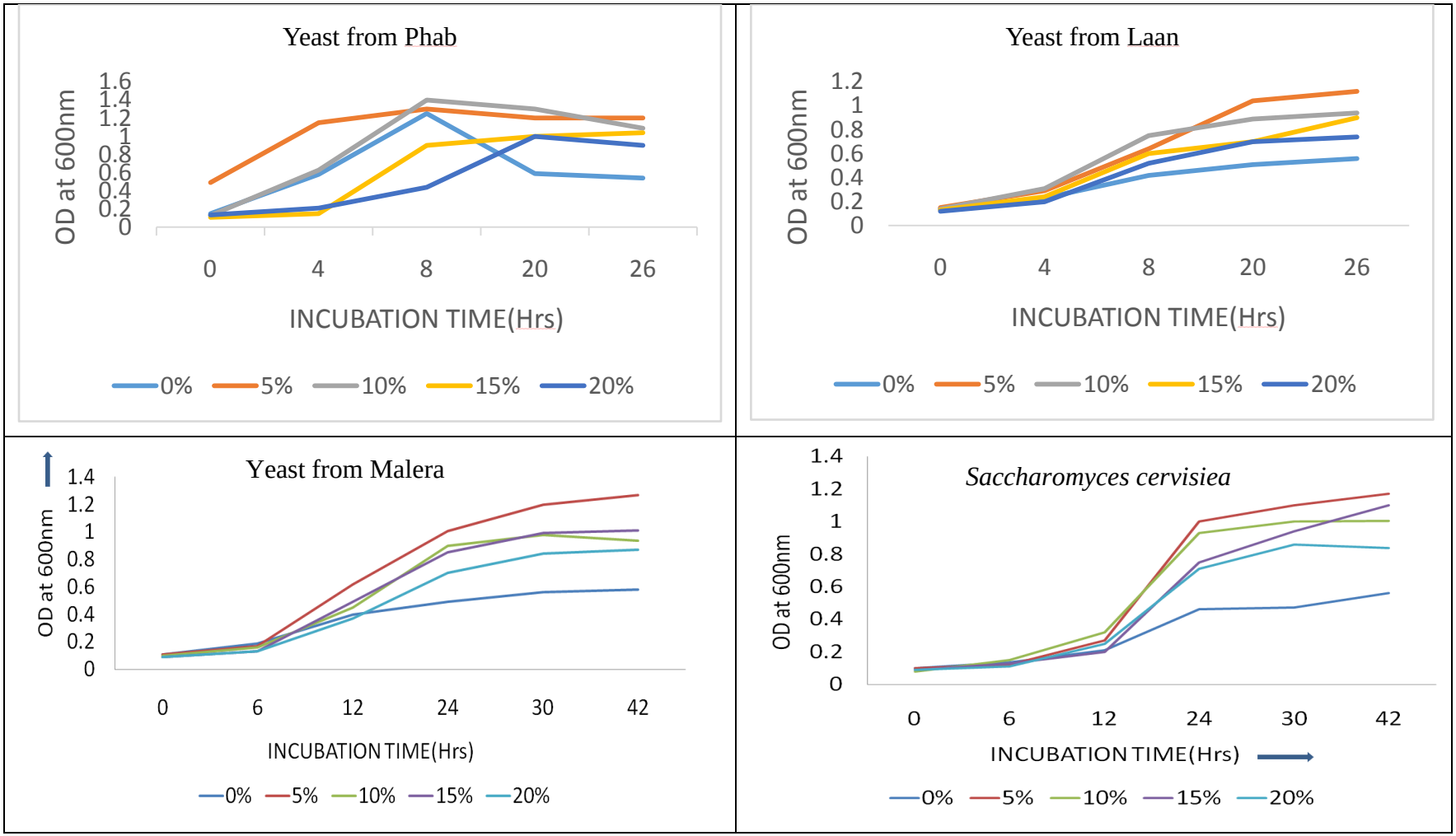

Figure 10: Graph showing Sugar tolerance of different cultures.

Range taken 0-20% with interval of 5%

- Yeast from *Phab*: Maximum growth at 10%
- Yeast from *Lahn*: Maximum growth at 5%,tolerance till 10% sugar concentration.
- Yeast from *Malera*: Maximum growth at 5%, tolerance till 15% sugar concentration.
- Saccharomyces cervisiea: Maximum growth at 5%, tolerance till 15% sugar concentration.

Yeast strain from *Malera* is competent to commercial yeast strain in terms of sugar tolerance as well with quicker adaption rate to high sugar concentrations (*Malera*-6 hours, Commercial strain- 12 hours) which will finally be converted to ethanol. Therefore, higher the adaptability, shorter will be ethanol production time and hence there can be more profits to the industry during the same time duration.

4.2.3 pH Tolerance:

Range taken 2-5 pH with interval of 1 pH

Acids give wines their characteristic crisp, slightly tart taste as well as help in the growth and vitality of yeast during fermentation and protect the wine from bacteria.

- Yeast from *Phab*: growth at pH4 and pH5 only.
- Yeast from *Lahn*: growth pH4 and pH5 only.
- Yeast from *Malera*: growth in pH4 and pH5 only.
- Saccharomyces cervisiae: growth in pH4 and pH5 only.

All strains are observed to show same results for pH tolerance. Lower the tolerance value of pH for a strain greater will be it capacity to resist increasing acidic conditions. Hence, it will be able to resist more ethanol without decrease in viability of yeast cells. Therefore, making the strain of more importance to industries.

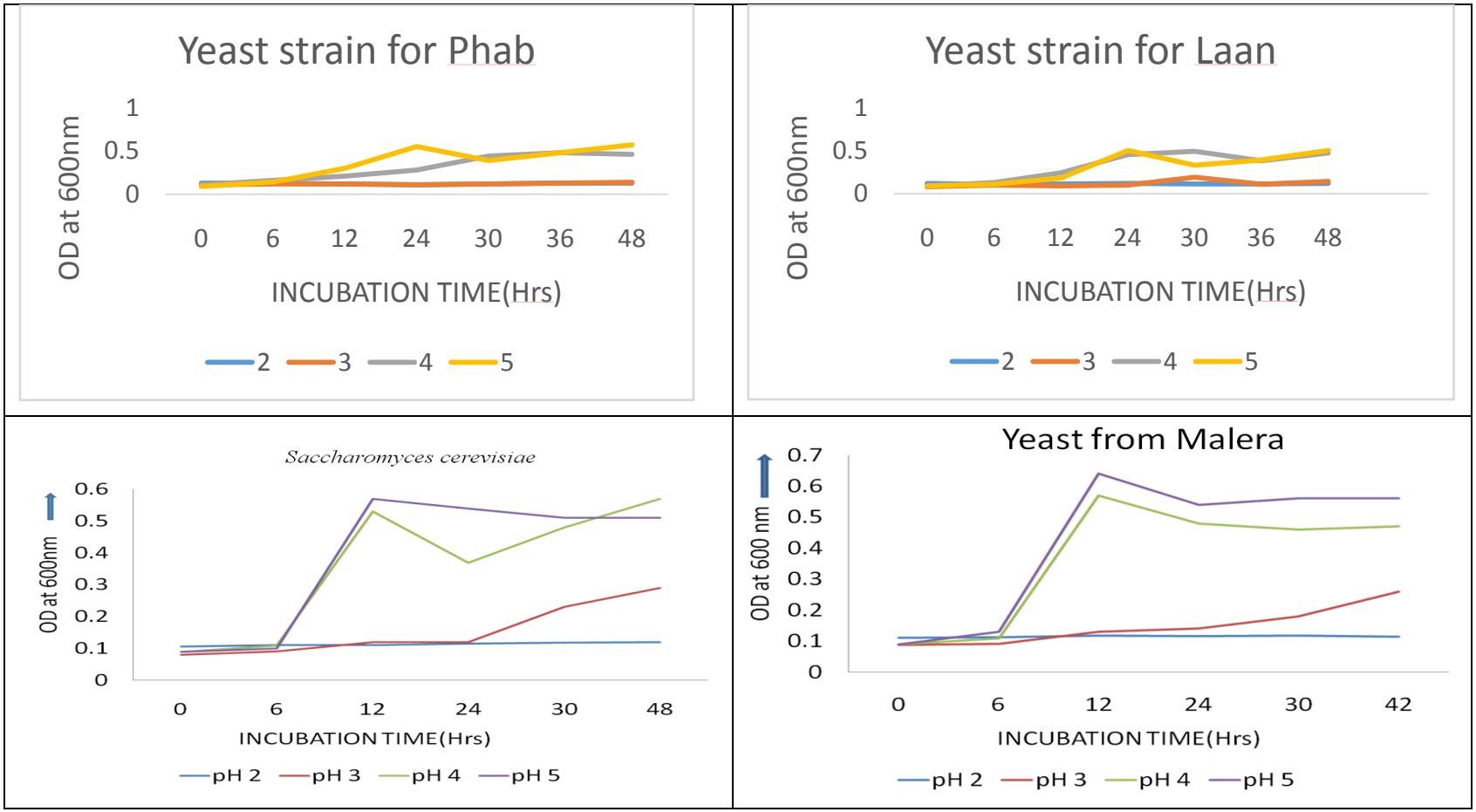

Figure 11: Graphs showing pH tolerance of different cultures.

4.3 Wine making:

4.3.1. Preparation of juice:

Juice extracted from the grapes was then passed through sieves of various sizes in order to remove grape skin from the juice in case skin was present in the juice. The juice was then pasteurized before the fermentation step in water bath at 60ºC for 15 min to decrease the microbial load in the fresh juice extracted.

Figure 12: Extraction of juice from grapes.

4.3.2.Setting of required brix degrees:

Refractometer is used to determine the total soluble solute in a liquid. The refractometer was initially wiped with ethanol and the zero level was set. Sample was initially checked for its TSS level and then sucrose was added to set 22ºbrix.

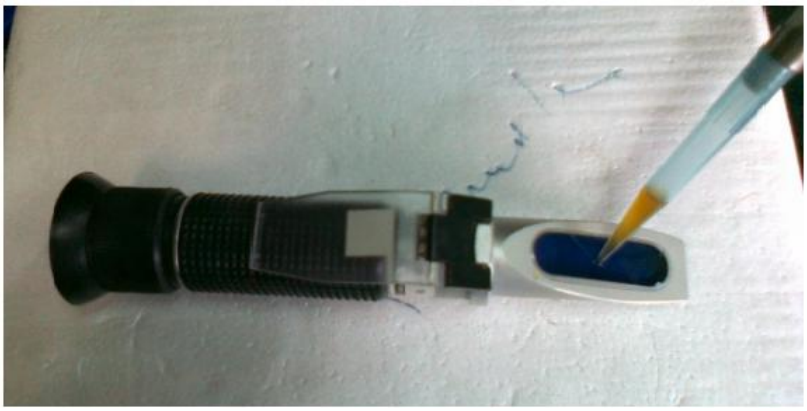

Figure13: Analysis of TSS with Refractometer.

5.3.3. Activation of yeast cell:

Figure 14:.Activation of different yeast starters in grape juice

Activation of juice at 37ºC without agitation was done till the point where air bubbles were visible. This activated juice acted as a seed culture for the remaining juice.

Small volume of juice is activated initially so that the yeast cells can adapt to the environment of juice initially and do not die due to environmental shock.

4.3.4: Filtration and Pasteurization:

Fermented juice was filtered using muslin cloth in LAF to improve its clarity.

Figure 15:Fermentation of grape juice

Figure 16:Fermented juice after pasteurization.

4.4 **Analysis of wine**

4.4.1. ABTS test:

Oxidative metabolism is very crucial for the survival of human cells. The risk of this activity leads to the production of free radicals which causes oxidative changes. Free radicals are linked with many pathological conditions such as diabetes, arthritis. Inhibition of the free radicals formed in the body is one of the important way to protect body from above serious diseases.

The antioxidant property potency of wine sample has been measured by ABTS assay.

ABTS is also frequently used by the food industry and agricultural researchers to measure the antioxidant property of food. This radical cation is blue in colour and absorbs light 734nm. During this reaction the blue ABTS radical cation is converted back to its colorless neutral form. This reaction may be monitored spectrophotometrically.

The blank was set at 1.0 for ABTS.

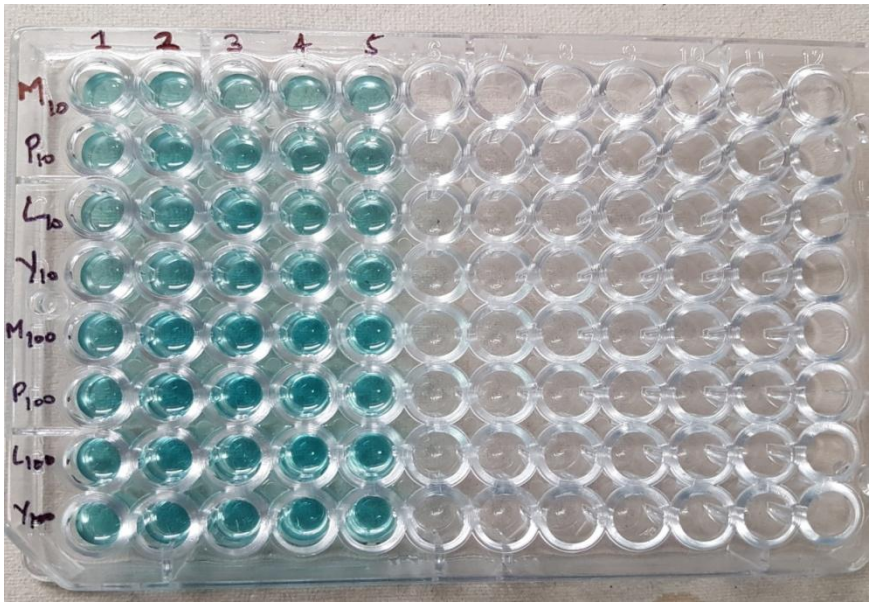

Figure 17: 96 Well plate with sample after 0.5 hr incubation

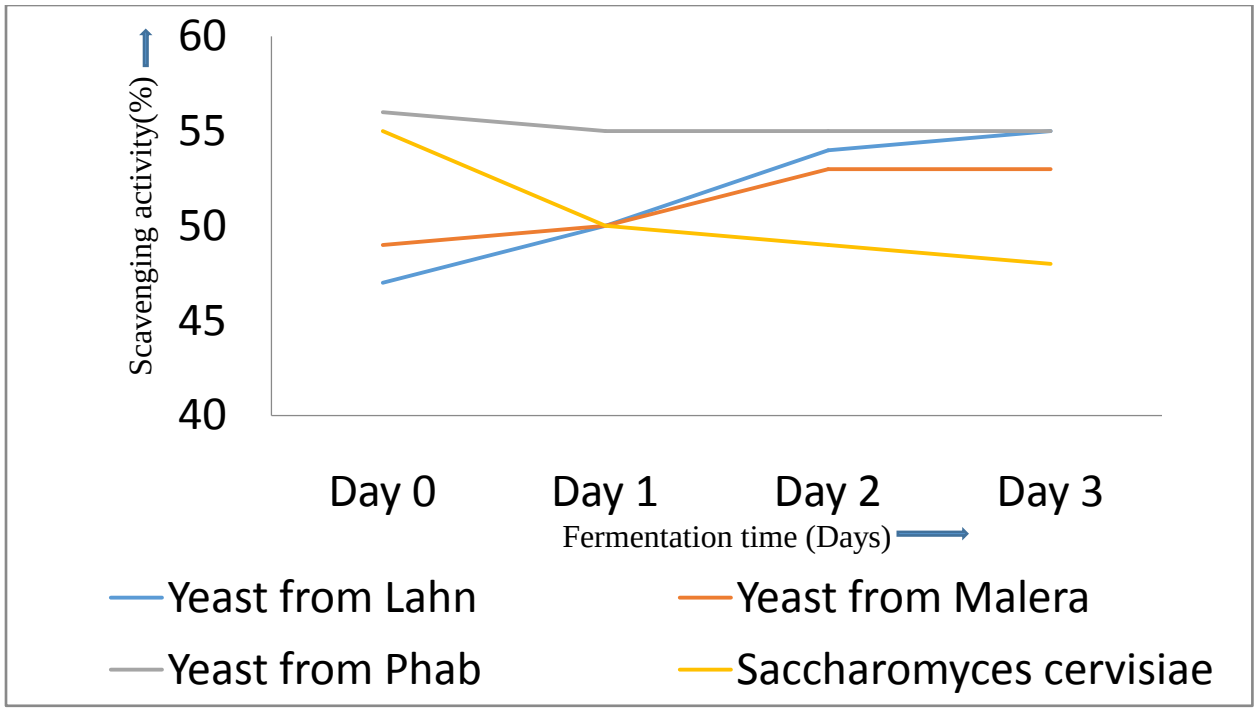

Figure 18: Graph representing scavenging activity of yeast strains

It is known fact that fermentation leads to increase in anti-oxidant activity of fermented juices which in turn indicates that phenolic contents also increases with fermentation.

- Yeast from *Lahn*: Antioxidant property continuously increased with fermentation time.
- Yeast from *Malera*: Antioxidant property continuously increased with fermentation time.
- Yeast from *Phab*: Anti- oxidant property was almost constant, there was no impact of fermentation on antioxidant property.
- *Saccharomyces cervisiae*: Anti-oxidant property was observed to decrease with increasing fermentation time.

It can be inferred that the indigenous strains of yeast used for characterization have higher anti-oxidant properties than the commercial strains. Hence, indicating they have better health benefits on human body as compared to the commercial yeast strain (in terms of their scavenging free radicals activity).

5.4.2. Total soluble solute:

This was used as a measure of the total soluble solids in the juice and was measured with the help of a hand-held refractometer. These soluble solids are primarily sugars; sucrose, fructose, and glucose. Citric acid and minerals in the juice also contribute to the soluble solids.

Brix is reported as "degrees Brix" and is equivalent to percentage.

- Constant ºBrix level indicated that the fermentation process had stopped.

Table5: Readings for Total soluble solute test.

Strains	Day 1	Day 2	Day 3	Day 4	Day 5
Lahn	15	8	7	6	6
Malera	15	11	7	7	6.8
Phab	19	8	6.1	6	5.8
S. cervisiae	15.5	8	7.5	7	7

From table it is inferred that:

- Yeast from *Lahn*- Fermentation stopped at Day 4
- Yeast from *Malera*- Fermentation continued till Day 5
- Yeast from *Phab*- Fermentation continued till Day 5
- *Saccharomyces cervisiae*- Fermentation stopped at Day 4

Total soluble solute of *Phab* was the least among other strains with a possible indication that maximum conversion of sugar to ethanol took place with yeasts from *Phab*.

Chapter-5
Conclusion

Conclusion:

Indigenous yeasts were isolated from traditional starter known as *Malera, Phab and Lahn* from wheat flour dough from the Himachal Pradesh region of North India was evaluated for its oenological and technological characteristics. These starters are traditionally used in fermented food items such as *Bhatoru, Siddu, Chilra, Marchu, Manna, Dosha, Pinni/Bagpinni, Seera* and several other beverages of the region. The properties of the isolated strains were compared with commercially available strain for technological parameters such as tolerance to sugar, alcohol, temperature and pH in Malt Extract Broth.

The newly isolated yeast strain "*Malera*" was found to be almost competent to the already existing commercial strain. The technological evaluation, and organoleptic properties of "*Malera*" indicates its potential for commercial brewing industry which may prove to be better than the existing strain used in terms of fermentation time. The use of this strain would help locals to gain incentives on traditional knowledge of the region of Himachal Pradesh. The strain, if found novel can also help gain IPR (Intellectual property right) benefits.

Chapter-6
References

References:

1. Savitri and BhallaTC, Characterization of *bhatooru*, a traditional fermented food of Himachal Pradesh: microbiological and biochemical aspects (2013);;*3 Biotech*; vol. 3; index 3; page no. 247-254.
2. S Kanwar S, Gupta M K, ChhayaKatoch, Kumar R and Kanwar P (2007); Traditional fermented foods of Lahaul and Spiti area of Himachal Pradesh (2007); *Indian Journal of Traditional Knowledge*; vol. 6; index 6; page no. 42-45.
3. Bhalla TC, Angmo K*;* Preparation of *phabs* – an indigenious starter culture for production of traditional alcoholic beverage, Chhang, in Ladakh (2014) ; *Indian Journal of Traditional Knowledge*; vol. 13; index 2; page no. 347-351.
4. Thakur N, Savitri and Bhalla TC; Characterization of some traditional fermented foods and beverages of Himachal Pradesh (2004); *Indian Journal of Traditional Knowledge*; vol. 3; index 3; page no. 325-335.
5. Sharma N, Handa S and Gupta A; A comprehensive study of different traditional fermented foods/beverages of Himachal Pradesh to evaluate their nutrition impact on health and rich biodiversity of fermenting microorganisms (2013); *International Journal of Research in Applied, Natural and Social Sciences*; vol. 1; index 3; page no. 19-28.
6. Molecular Mechanisms in Yeast Carbon Metabolism The second completely sequenced yeast genome came 6 years later from the fission yeast Schizosaccharomycespombe, which diverged from S. cerevisiae probably more than 300 million years ago
7. *Kurtzman CP, Fell JW (2006). "Yeast Systematics and Phylogeny—Implications of Molecular Identification Methods for Studies in Ecology". Biodiversity and Ecophysiology of Yeasts, The Yeast Handbook. Springer.*
8. *Hoffman CS, Wood V, Fantes PA (October 2015). "An Ancient Yeast for Young Geneticists: A Primer on the Schizosaccharomycespombe Model System." Genetics.* ***201*** *(2): 403–23. doi:10.1534/genetics.115.181503 PMC 4596657 . PMID 26447128.*
9. *Kurtzman CP, Piškur J (2006). "Taxonomy and phylogenetic diversity among the yeasts". In Sunnerhagen P, Piskur J. Comparative Genomics: Using Fungi as Models. Berlin: Springer. pp. 29–46. ISBN 978-3-540-31480-6.*

10. *Yong E (16 January 2012). "Yeast suggests speedy start for multicellular life". Nature News. Nature.*
11. *Kurtzman CP, Fell JW (2005). in: The Yeast Handbook, Gábor P, de la Rosa CL, eds. Biodiversity and Ecophysiology of Yeasts. Berlin: Springer. pp. 11–30. ISBN 978-3-540-26100-1.*
12. *Walker K, Skelton H, Smith K (2002). "Cutaneous lesions showing giant yeast forms of Blastomycesdermatitidis". Journal of Cutaneous Pathology.* ***29*** *(10): 616–618. doi:10.1034/j.1600-0560.2002.291009.x. PMID 12453301.*
13. *Legras JL, Merdinoglu D, Cornuet JM, Karst F (2007). "Bread, beer and wine: Saccharomyces cerevisiae diversity reflects human history". Molecular Ecology.* ***16*** *(10): 2091–2102. doi:10.1111/j.1365-294X.2007.03266.x PMID 17498234.*
14. AakritiGuleria, Production of Grape wine by the use of yeast, *Saccharomycese cerevisiae*, Abhilashi Institute of Life Sciences, Department of Microbiology, Mandi - 175008, Himachal Pradesh, India, vol. 3, issue-6, June 2014.