CULTIVATION AND PROPAGATION OF BEER YEAST FROM THE STARTER CULTURE OF *Saccharomyces cerevisiae*

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Abstract

The quality and the high level production of beer are related to different types and races of yeast cultures. Some characteristics such as rate of multiplication, speed of fermentation and aroma are directly affected by the purity and type of the yeast. As the matter of fact our study is focused on cultivation of pure culture of *Saccharomyces cerecisiae.*
Analyses are done in a brewery and have consisted on yeast cultivation; a single cell is first selected and used to propagate a pure strain into an agar plate (or Petri dish). The pure culture is stored under strict conditions, in order to maintain the same racial characteristics of it. For propagation and cultivation processes we have used the Carlsberg flasks, and the pure yeast culture is then inoculated in the sterile wort. During fermentation starter culture is transferred to the vessels which change in a progressive way their size. These steps are followed by microbiological and yeast multiplication analysis. The propagation vessels are closed and equipped with heating and cooling coils, which are used for sterilizing and cooling the wort. After the cultivation of the right starter and the propagation of yeast we have noticed very good beer characteristics, good aroma and much more flavor than before. The experimental study in cultivation of pure yeast culture is done at special microbiological laboratories.

Key words: beer yeast, *Saccharomyces cerevisiae*, propagation, starter culture, Carlsberg flask.

Introduction

The quality of the beer yeast will have a significant impact on the final product, so it will be treated carefully and looked after. Changes in the integrity or health of the yeast may impact on the characteristics of the package beer.
Most of brewers collect yeast from one fermentation and reuse it in subsequent fermentations. Brewers does not use yeast after a certain number of reuses and replace this with propagated yeast.
The development of pure yeast strains and their importance in the brewing process has been going on for over a century and is still an active area of research. The first yeast propagation plant for brewing has been attributed to Hansen and Kuhle and was based on the principles of batch propagation. They described the first techniques for isolating single yeast cells and propagating them to a larger scale.
Since those times for yeast propagation have been tried many variations ranging from batch systems to continuous systems.
The main objective of yeast propagation is to produce sufficient yeast for a full-scale brewery production. The yeast should be produced in a short time as soon as possible.
The propagated yeast should have the desired physiological condition to develop the desired fermentation performance. This should be consistent from propagation to propagation.
When yeast is inoculated to wort or media, it goes through 5 basic stages of growth.

1. Lag phase –period of adaptation
2. Accelerating growth phase – initiation of yeast growth
3. Exponential phase – rapid yeast growth, doubling every 2-3 hours
4. Decelerating growth phase – nutrient supply depleted.
5. Stationary phase – no yeast growth, cell viability begins to decline

Biomass productivity per unit time is highest during the logarithmic phase of growth and lowest during the stationary phase. Work by Hulse *et al*. confirmed that the optimum time to transfer yeast from one stage of propagation to the next is in the late logarithmic stage of growth, before the beginning of stationary phase. This offers time advantages so the next lag phase of growth is minimized and the propagated yeast is not subjected to any nutrient stress.
Research into yeast propagation has been concentrated on increasing the biomass productivity of the systems by focusing on the role of nutrients and improving aeration, with some work into alternative systems such as fed-batch and continuous systems.
There are several factors that influence both yeast growth (and fermentation) and should be considered during the propagation and maintenance of yeast. The most important factors are oxygen, pH, temperature, and wort composition.
Von Nida further claimed that the yeast crop produced by means of an aerobic propagation process has a lower tendency to autolysis. This will have obvious product benefits in terms of taste and head.
The development of pure yeast strains has a great importance in brewing process, in order to produce beer with a uniform high level of quality, it is necessary to work with pure yeast strains, since aroma, rate of multiplication, and speed of fermentation are characteristic properties of a particular type of yeast. There are a number of characteristics that differ between types and races of culture yeast.
The propagated yeast should be free from contaminants. The consequences of contamination with bacteria and wild yeast can affect the quality of the beer. Most contaminants will produce off-flavors, acids and non-desirable aromas.

Material and Methods

Propagation:

The selection of yeast for good propagation and beer fermentation was achieved by applying the following criteria:

- adequate yeast cell counts to achieve the desired pitching rate in fermentation
- good viability (>95%),
- hygiene,
- good vitality or metabolic activity with high glycogen and sterols levels.

To ensure these, the propagation was taking place in sterile wort in presence of oxygen. The temperature of propagation was between 20-25°C to avoid a stressful environment, especially for lager strains.

The process initially began in the laboratory when cultures were taken from the "working" master culture and grown in a progression of fermentations of increasing size until enough yeast is produced to transfer to the propagation plant. The number of transfer steps in the laboratory varies according to the final weight of yeast required for the propagation plant. Of course, the more transfers, the greater the risk of contamination.

1. Laboratory propagation

The aim of the laboratory phase propagation is to generate a pure yeast culture of sufficient size to provide an adequate pitching rate for the first stages of brewery propagation.

We started with some yeast colonies from a agar plate that was cultivated and well controlled before using it. We selected a yeast colony that was inoculated in 10 ml sterile wort. When the fermentation began, this starter culture was transferred to progressively larger vessels.

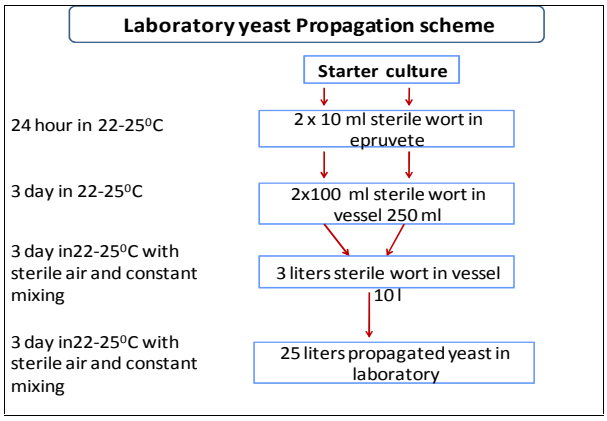
10 ml → 250ml wort (500 ml vessel) → 3Lwort (10 L vessel) → 16 L wort (25 L vessel)

It was important to transfer the yeast while it was in exponential phase, to keep the yeast active and avoid potential contamination. During each transfer we have conducted some different analyses, where the most important of them were microbiological control.

For propagation and yeast cultivation was used the Carlsberg flask. The terminal laboratory culture was held within a container, which allows transfer to the brewery propagation vessel under aseptic conditions. During the laboratory propagation was used standard microbiological apparatus and skilled personnel.

In the initial stages we used artificial media such as yeast extract, peptone, and glucose. After that we add sterile wort (the same wort of beer fermentation) that was sterilized by autoclaving prior to use in the other laboratory stages.

Laboratory propagation is done as is shown in the scheme below:



The terminal laboratory stage requires a stainless steel flask (keg) with a capacity of approximately 25 liters. The inoculated culture was transferred to the flask. After inoculation, the flask was continually aerated with air or pure oxygen. Oxygen transfer rates were improved by constant agitation using a powerful magnetic stirrer. The released gas was vented to atmosphere via another microbiological grade filter.

2. Brewery Propagation Plants

The propagation plant usually is equipped with two or more closed stainless steel vessels of increasing volume, which are usually situated in a separate room to minimize risk of contamination.

All propagators, pipe work and valves connecting propagation vessels and the rest of the brewery are built to the highest hygienic standards.

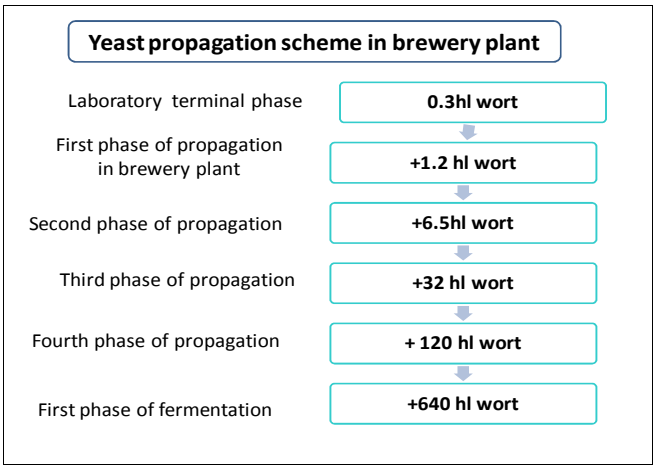
As we mentioned above microbiological sensitivity of the process requires the wort sterilization. Often this was achieved by using a separate sterile wort holding tank from which wort was taken to feed the propagation vessels.

All brewing yeast strains have optimal growth temperatures of around 30°C. Commonly a relatively high temperature may be used in the first vessel followed by a gradual reduction in temperature at each subsequent stage with the terminal propagation being performed at the same temperature as the first fermentation (Maule, 1979).

The reasons why we propagate at relatively low temperatures and limiting the availability of oxygen are:

- First, this ensures that the yeast suffers no thermal shock.
- Second, high propagation temperatures and excess oxygen favor elevated levels of higher alcohols, acetaldehyde, diacetyl and reduced esters.

Brewery propagation was realised as is shown in the scheme below:



Brewing yeast that was developed on wort gravity (10-12 ^{0}Plato) under continuously aerobic conditions and temperature within the range 20 to 25°C it yield a terminal yeast count approximately 200-300 x $l0^{6}$ cells/ ml within 36-48 hours.

This process was terminated when the maximum cell count was achieved and aeration was discontinued, there was no opportunity to occur the transition between fermentative and oxidative physiology.

A typical cylindroconical fermenter of 1500 hl capacity was serviced by a 100 hl propagator vessel. After this procedure was achieved a terminal count from propagator of 250 x $l0^{6}$ cells/ ml and a pitching rate in the first fermentation of 15 x $l0^{6}$ cells /ml.

As may be seen, the entire propagation process from laboratory to first fermentation was lengthy as was taking a matter of weeks to be completed.
As the propagation process was completed we inoculate the yeast in a fermentation tank. The pitching rate for brewer's yeast will depend on the original gravity of the wort to be inoculated (1 million viable of propagated or recycled yeast cells are inoculated per Plato degree).
The frequency of introduction of newly propagated yeast into the brewery is a decision for the individual brewery since there are no immutable rules. However, in our brewery we allow the use of 5-8 generations before introducing new yeast.

Results and discussions

Yeast can influence the taste, flavor, bouquet, and even the color of beer. It makes this by secreting a variety of compounds at very low levels.
The propagation and maintenance of yeast adds the level of control to the brewing process.
Three major things must be considered when choosing a method of yeast storage. These are yeast strain purity, viability and genetic stability. Each of these differs depending on the method of preservation.
The standard method for maintaining yeast and bacteria is on some type of solid media either in the form of plates or slants. Agar is typically used as a solidifying agent and is added at a concentration of 1.5-2% (1.5-2.0 grams per 100 ml liquid). The base media can be wort or one of the laboratory media. Agar is insoluble in wort or media and needs to be boiled for a few minutes to dissolve. After pouring plates or slants it is important that they be sufficiently dried at room temperature (2-5 days) before using them. Otherwise condensation may form on the sides of the tube or petri plate during storage which can lead to contamination especially by mold and fungus.
It is important to point out that the media used for storage should be sterile. That means all micro-organisms including spores are destroyed. This is done by heating in an autoclave or pressure cooker for 15-30 minutes at 15 psi.
Monitoring of yeast health was realized through microscopic appearance examination, by using the brewery standard dyes methylene blue or methylene violet, viable and non-viable cells are distinguished in a given yeast population.
By using a special microscope slide, known as a haemocytometer, we counted the number of cells in a given suspension. Microscopic examination of our yeast and beer also enable us to detect any substantial contamination which may not otherwise be evident.
Even in the best-managed brewery, the production environment provides opportunities for the introduction of contaminants to yeast. These may be in the form of bacteria or wild yeast. The consequences of contamination with wild yeast are significant, resulting in process changes (flocculation, fining) or flavour changes (phenolic, medicinal character).
The standard laboratory media for growing and maintaining yeast included YPD, potato dextrose, and sabouraud dextrose. These media are designed for laboratory yeast and do not contain a complex source of fermentable sugars.

Microbiological analysis of propagated yeast:
Through this study we aim to determine the total microflora of propagated yeast and so the beer characteristics that was fermented with this yeast. Microbiological control was accomplished by analyzing yeast directly in propagated vessels. The results of microbiological analyses are shown by graphics, where are given the relation of logarithm of (N). N is the number of microorganisms.

Microbiological analyses of yeast were performed in simple and selective mediums to distinguish bacteria, wild yeast and moulds present in fermented yeast including: the Czapek, yeast extract agar (CYA), malt extract agar (MEA), potato dextrose agar (PDA), plate count agar (PCA), the McConcey agar, MRS (De Man, Rogosa and Sharpe), W-L differential medium, NBB-B and NBB-A (Ref. 6). Plates were incubated at 27(± 2) °C for 2 till 7 days for developing of moulds.
The samples was taken directly from vessels that were used for yeast propagation and storage prior it was pitched to fermentation tank.
In Figure.1 are given the results of total microorganisms of yeast propagated.

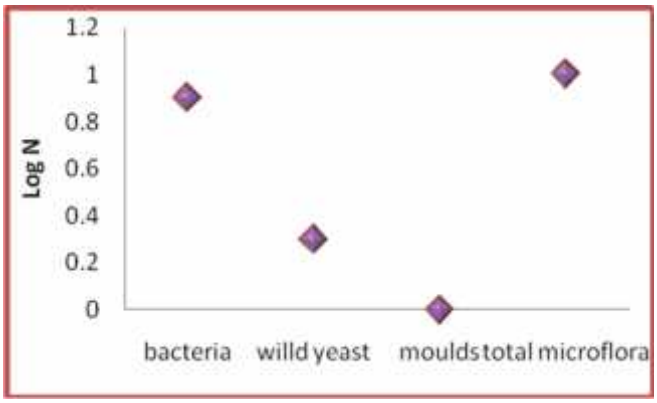


Figure1. Microflora of yeast propogated

It has been noticed from the microbiological analyses that the contamination is mainly bacterial and it is in very low level, so we can use this yeast in fermentation proces.
Bacterial contamination of yeast beer was found to be a consequence of wort contamination, poor hygiene conditions of the environment, the transfer of yeast to larger vessels and pipe lines systems.
In Figure 2 is shown total microflora of beers fermented with some different kinds of yeast including our propagated yeast.

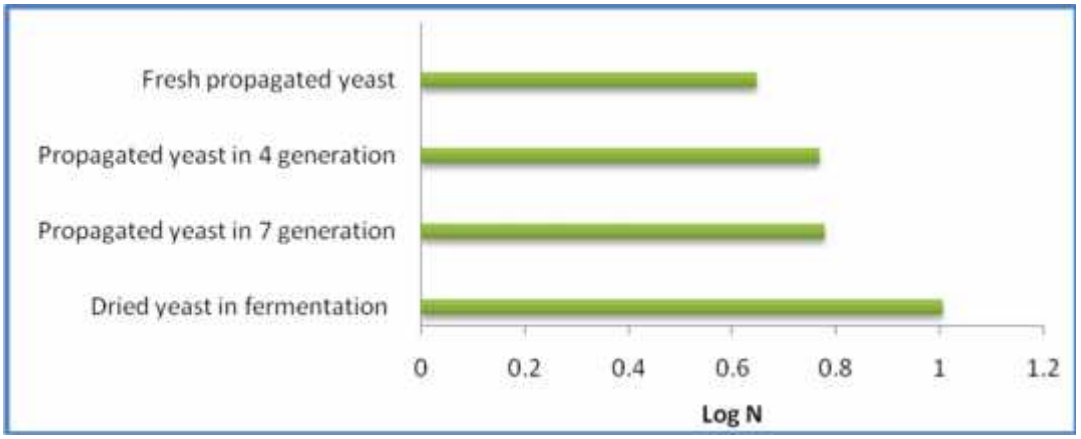


Figure 2. Total microflora of beers fermented with different yeast.

As we noticed from the figure the beer fermented with fresh propagated yeast has the lowest microbiological contamiantion compared with other beers.
After the cultivation of the right starter and the propagation of that yeast and after microbiological analysis, microscopic controll, counting number of cells and viability, there were noticed good beer characteristics.

In the tables below there are given two cases of beer fermentation. In the first table are shown physico-chemical and microbiological results during different times of fermentation carried with propagated yeast.

Table.1.

Fermentation carried with propagated yeast in brewery					
Physico-chemical and microbiological analyses	24 hours	3 day	7 day	In cooling	Maturation phase
pH	5.1	4.7	4.2	4.2	4.2
Temperatura (^{o} C)	9	10	7	1	0
Extract (Plato degree)	10.4	6.5	2.5	2.2	2
Diacetyl (mg/l)			0.075	0.052	0.048
Acidity (g lactic acid /100 ml beer)				1.8	1.8
Total microflora (cells/ml)	20	15	10	7	3

In the second table are shown physico-chemical and microbiological results during different times of fermentation carried with yeast that is not propagated in brewery.

Table. 2.

Fermentation carried with non propagated yeast in brewery (another yeast)					
Physico-chemical and microbiological analyses	24 hours	3 day	7 day	In cooling	Maturation phase
pH	5	4.6	4.25	4.2	4.2
Temperatura (^{o} C)	13	14	10	2	0
Extract (Plato degree)	10.2	5.5	2.5	2	2
Diacetyl (mg/l)			0.12	0.09	0.08
Acidity (g lactic acid /100 ml beer)				2	2
Total microflora (cells/ml)	55	48	40	32	28

As we see from those tables beer fermented with our propagated yeast has good parameters compare with the other beer. It has lower diacetyl values and it is almost free of contamination.

In order to provide a high performance, a good quality and a shelf life of the final product we use the propagated yeast free of contaminants.

Conclusions and Recomandations

Based on the above information, a yeast starter should be composed of a 1.040 gravity wort. It should be aerated well before adding yeast. Once the yeast is added it should be kept at room temperature and shaken as often as possible (or better yet, constantly stirred).

All media used to store brewing yeast (slants, plates, etc.) should contain yeast nutrients, some malt, and should have an acidic pH.

The physiological condition of the yeast must be consistent and appropriate for subsequent fermentation.
The cycle time of propagation should be as rapid as possible, both for economy and to minimize the risk of contamination, and should be used the fewest possible number of vessels.
As the conclusion the reasons why brewers use propagating yeast are the following points:

- permit the use of pure cultures
- accommodate changes in behavior as a result of cropping practices
- prevent deterioration in yeast fermentation performance in subsequent fermentations
- eliminate the presence of contaminants
- reduce the risk of mutations

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