

Brewing yeast selection

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RIGHT:
Fig. 1. Progress of a typical ale fermentation (top yeast), showing the best time (arrow) for 'skimming' the yeast head as inoculum for the next fermentation. The rapid fall in the number of yeast cells in suspension at the end of the period of active fermentation is caused by flocculation of the yeast.

● The brewing process

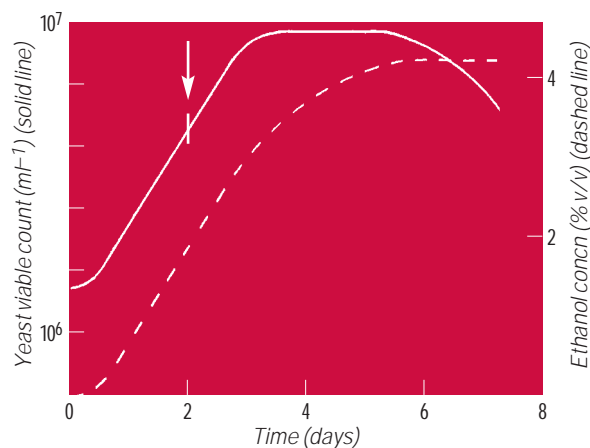
Perhaps it is best to start with a brief outline of the brewing process. The brewing yeast *Saccharomyces cerevisiae* is unable to utilize the starch of barley, so the grain is first germinated and the resulting malt is extracted with hot water to yield wort containing fermentable mono-, di- and trisaccharides and other yeast nutrients. The wort is boiled with hops or, in the past, other flavourings, cooled and inoculated with a suitable yeast culture. After a period of maturation and clarification the product should be a palatable beer.

● Selection in ancient times

Brewers have been selecting yeasts for thousands of years. The first beer fermentations must have been carried out by naturally occurring yeasts. That practice still survives in the production of Belgian lambic beer, for which airborne yeasts and bacteria and the resident microflora of the wooden wort cooler and fermentation vessels provide the inoculum. The ancient brewers must have noticed that some batches of naturally inoculated beer were better than others. They must also have realized that collecting the frothy head from the surface of the fermenting beer and adding it to the next batch of wort gave a much faster fermentation. Therefore, the first (unrecorded) instance of yeast selection was to combine these two observations and use the yeast head from a particularly good fermentation to repeat the favourable results with the next batch. Long before the requirements could have been expressed in microbiological terms, brewers instinctively recognized the characteristics of a good brewing yeast (Table 1).

● Flavour and flocculence

Another type of selection occurs during each fermentation. Yeast multiplies about 10-fold during a beer fermentation, so only about one-tenth of the yeast growth has to be collected from each fermentation to continue propagation indefinitely. Traditional brewing yeasts are unlikely to be pure cultures, so there is also the requirement to maintain a constant composition of the



mixture. Different strains of yeast vary slightly in their production of the numerous metabolites that contribute to beer flavour: at least 400, according to one estimate. It may not have been so important in the distant past, but now customers expect a particular brand of beer to have a consistent flavour. If the yeast head is collected at the same stage of each successive fermentation, the various strains which make up the yeast culture will be continued in approximately the same proportion, and therefore it is reasonable to expect consistent flavour production. Typically, one strain of the yeast population may accumulate in the head in the early stages of the fermentation, another strain rises later and a third strain predominates later still. Yeast head must be skimmed off several times during the fermentation to prevent it collapsing back into the beer and creating off-flavours, but only part of the recovered yeast is re-used for subsequent fermentations (selection!). After 48 h of fermentation (Fig. 1), using the second (middle) skimming from each successive fermentation gives the best chance of maintaining a constant balance of strains. Not only do yeasts vary in flavour production, there is also a variation in flocculence. Flocculation of yeast is the spontaneous aggregation into clumps which settle increasingly rapidly out of suspension (see Fig. 1). Too early flocculation brings the fermentation to a premature end; but non-flocculent yeasts remain in suspension and have to be removed by expensive centrifugation or filtration. Therefore the correct degree of flocculence developing late in fermentation is essential for economical production of bright beer.

● Probably the best yeast in the world? Lagers and ales

But, several hundred years earlier, perhaps the most important instance of yeast selection in the entire history of brewing was achieved in Bavaria. In Britain, enthusiasts become excited about the differences between 'ale' and 'lager'. In almost every other country of the world, the words 'beer' and 'lager' are synonymous. It is amazing that a speciality beer of an unknown Bavarian

Table 1. Essential properties of brewing yeast

a	Consistent production of flavour and aroma metabolites
b	Rapid fermentation
c	Efficient fermentation (maximum yield of ethanol, minimum production of new yeast biomass)
d	Tolerance to the inhibitory effects of wort and beer (osmotic stress of initial sugar, toxic effect of final alcohol and CO ₂)
e	Suitable flocculation and sedimentation properties at the end of fermentation (and for 'top fermentations', head formation)
f	High final viability for inoculating (pitching) the next fermentation
g	High genetic stability over successive fermentations

monastery has become the worldwide standard beer. Previously, beer had been fermented by 'top yeasts', harvested from the surface of the fermentation to propagate the next fermentation. The 'bottom yeast' of the lager fermentation did not form a true yeast head, only a foam which contained too little yeast to seed a subsequent fermentation. So settled yeast had to be recovered from the bottom of the vessel at the end of fermentation. The name lager came from the storage (*lagern* in German), really a secondary fermentation at low temperature, for improvement of flavour and CO₂ content. The other technical advantages of the production of lager are irrelevant to a discussion of yeast selection, but the fame of the initially local beer soon spread. First, the yeast was stolen by Czech brewers to begin brewing at Pilsen (which, perhaps unjustly, gave its name to that type of beer) and later by one of the Jorgensen family of the Carlsberg company. There, the pioneer yeast taxonomist E. C. Hansen, a contemporary of Pasteur, first isolated pure yeast cultures and recognized the 'top yeast' of the traditional beers of Belgium, Britain and Germany as a different species, *S. cerevisiae*, from the 'bottom yeasts' of the Bavarian and Czech beers which, in a shrewd career move, he named *Saccharomyces carlsbergensis*. Pure cultures of *S. carlsbergensis* were then exported, creating the worldwide production of the 'pilsener' type of beer.

● Current techniques for yeast selection

Much later, *S. carlsbergensis* was recognized as virtually indistinguishable from the wine yeast *S. uvarum*. So the original Bavarian isolate may have been a chance contamination from local wine production, and subsequently propagated because of its desirable flavour characteristics. Now, however, for improvement of a brewing strain the microbiologist must undertake some deliberate manipulation of the yeast. With its virtually unlimited possibilities, genetic engineering may spring to mind as the first means of selection. In fact, that would be the last choice, not least because of the potential impact on sales of beer made with such yeast. The techniques for genetic improvement of yeast are listed in the usual order of preference (Table 2).

Any type of genetic manipulation involves plating out the recovered hybrids or mutants, followed by trial small-scale fermentations with cultures from individual colonies. Therefore, it is sensible to start by screening the existing culture to discover if a strain of the desired properties is already present as part of the mixture. An important instance of this method was the selection of non-head-forming variants of the existing yeast at the time of change from traditional open rectangular tanks to modern enclosed cylindro-conical fermenters (Fig. 2). In the latter, not only is it technically impossible to use a skimming system for recovery of yeast, but the vigorous circulation by rising CO₂ and downward movement by

Table 2. Techniques for genetic manipulation of brewing yeast

a	Screening of existing culture and selection
b	Mutation (usually UV) and selection
c	Hybridization (crossing haploid mater cultures, or sphaeroplast fusion) and selection
d	Recombinant DNA technology and selection

Table 3. Possible genetic improvements to industrial yeasts

Group 1: conferred by introduction of the appropriate gene

a	Hydrolysis of starch and dextrans
b	Hydrolysis of cereal β-glucan
c	Increased rate of fermentation
d	Optimal flocculation properties
e	Acetolactate decarboxylase
f	Hydrolysis of cellulose/cellobiose or lactose (not applicable to brewing yeast, but important for economical production of industrial alcohol)

Group 2: economically important, but biochemical and genetic basis not yet defined

a	Reduced requirement for dissolved oxygen
b	Tolerance of high initial sugar concentration and high final ethanol concentration
c	Ability to ferment at higher temperature

wall cooling creates even more head than in rectangular vessels. The amount of surface froth shown in Fig. 2 is typical of a non-head-forming yeast. If the traditional top yeast in the rectangular vessel at the top had been used in the large fermenter, at least half of its volume would have been wastefully filled with yeast head. A non-head-forming mutant of exactly the same flavour characteristics as the original yeast is preferable to the use of antifoam. It is true that lager yeasts could have been used, but these would have caused an obvious difference in flavour.

Mutagenesis, usually by irradiation, works by inactivating one or more genes. In any genetic manipulation it is essential to preserve the valuable existing properties of the brewing yeast and there is a distinct possibility that a mutation will delete some essential characteristic. Chemical treatment may be inadvisable because of the risk of residual mutagen, but UV irradiation has occasionally resulted in selection of an improved strain.

Successful results have been achieved by the chance deletion of a suppressor gene; alternatively, back-crossing the mutant with the original strain has added the desired improved characteristics. Certainly, there are possible improvements which require deliberate introduction of new genetic characters (Table 3).

In the life cycle of *S. cerevisiae*, discovered in 1935, the diploid nuclei of vegetative cells become haploid

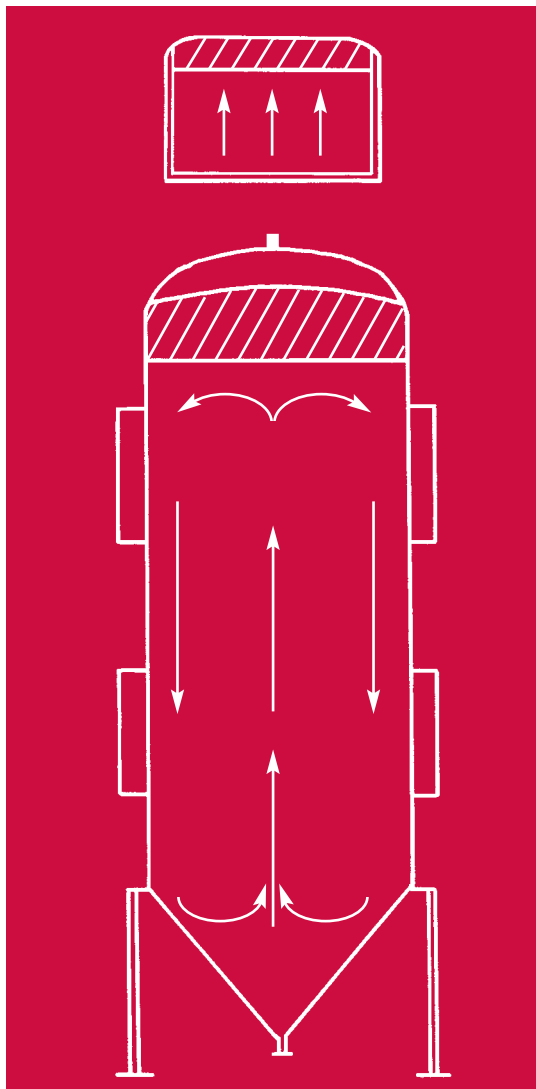


Fig. 2. Comparison of a traditional open rectangular vessel and a modern cylindro-conical fermentation vessel, drawn to the same scale. The shaded section represents head or froth above the fermenting beer; the arrows show the direction of currents within the fermenter.

on sporulation; micro-dissection of spores and germination in the absence of the opposite mating type provide haploid mater cultures. This resulted in *S. cerevisiae* becoming a useful eukaryotic model for genetic research. Unfortunately, most brewing yeasts have lost the ability to form spores. Over centuries, or millennia, of propagation in a rich culture medium, spores were unnecessary; also, with the inevitable genetic exchange during such prolonged intensive cultivation these industrial yeasts are no longer diploid, but of complex ploidy which would inhibit sporulation even if all of the necessary genes were still present. However, sphaeroplast fusion does

not require maters and successful hybridizations have been achieved by that method.

● Desirable genetic improvements

Introduction of the ability to ferment higher oligosaccharides than maltotriose has been achieved by methods (c) and (d) of Table 2. Since these dextrans constitute at least 20% of a normal wort, there is potential for additional alcohol yield from the same amount of malt, or production of 'light' or 'dry' beers after utilization of the dextrans.

Another potentially useful ability is hydrolysis of cereal β -glucan which, in excess, causes haze in the beer and by its viscosity, filtration problems. Improved filterability and destruction of glucan haze are valuable properties of that modified yeast. Only a single new gene would be required in each case, for either amylase or glucanase, but in most countries the same effect can be achieved legally and more easily by adding the appropriate enzyme to the beer. For some reason there is popular revulsion to genetically modified organisms but, except among the most dedicated beer enthusiasts, there seems to be little objection to the use of enzymic processing aids.

The economic benefits of faster fermentation are obvious and can be achieved by increased content of *MAL* genes for maltose transport and hydrolysis, although other methods may also be effective. Flocculation, the spontaneous aggregation of yeast cells,

is important for clarification of beer, but must be genetically programmed to occur only at the end of fermentation. Flocculation too early causes clumps of yeast cells to settle before fermentation is complete. Non-flocculent yeasts are also troublesome: they must be removed by centrifugation.

Introduction of acetolactate decarboxylase to brewing yeast reduces production of diacetyl, the buttery flavour which is generally regarded by professional brewers as an objectionable off-flavour, although at low levels it does not seem to annoy the general public. Acetolactate is a by-product of biosynthesis of isoleucine, leucine and valine, and if released from yeast cells is spontaneously oxidized to diacetyl. If acetolactate is no longer excreted there is no longer a diacetyl problem. Genetic manipulation to introduce appropriate *MAL*, *FLO* and *ILV* (isoleucine, leucine and valine) genes, therefore, has beneficial effects on beer quality.

S. cerevisiae is not a true facultative anaerobe like *Escherichia coli*. Although capable of fermentation, it is unable to grow indefinitely without oxygen. For flavour reasons, mainly related to diacetyl production, oxygen can be provided only in the early stages of fermentation. If other brewing qualities are acceptable, the best brewing yeasts have the lowest requirement for dissolved oxygen in the wort at the time of pitching.

Osmotic tolerance is important for the modern technique of high-gravity brewing, whereby plant capacity can be doubled by brewing double-strength wort, fermenting to double-strength beer and diluting to sales strength as the final stage of the process.

Ability to ferment at higher temperature is probably not relevant to beer production, but is important for distilled alcohol: the higher the fermentation temperature, the less energy is required for distillation. The ultimate goal would be distillation without any heat energy input, if the fermentation itself generated sufficiently high temperature that application of a partial vacuum would suffice.

All of the improvements listed in Table 3 are technically possible and many have already been incorporated into production strains of yeast. Using the others is dependent on public opinion rather than brewing technology, so it is impossible to predict how yeast selection will develop in the future.

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Further reading

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