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# ATTENUATION OF BEERWORT BY STRAINS OF *SACCHAROMYCES CEREVISIAE* HANS.

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With a view to select a strain of yeast which will be suitable for the manufacture of beer under local conditions, different strains of yeast, *Saccharomyces cerevisiae*, were obtained from different sources. These strains were plated out in beer wort-agar medium and pure cultures from single colonies were established. A comparison of the degree of attenuation amongst the different strains of yeast was made. It has been shown that a single strain of pure yeast is more suitable for beerwort fermentation than mixed races.

## INTRODUCTION

The use of selected strains of *Saccharomyces cerevisiae* in fermenting beerwort has long been adopted in all breweries of the world. The selection of such strains is based principally upon the ability of the yeast in question to break down the constituents of the wort during fermentation. In order to study the activity of yeast strains on the medium of beerwort in which they are allowed to grow and ferment, the use of a wort of almost uniform composition is essential. The quality of the fermentation products depends upon the nature of the medium and the manner in which the yeast cells behave. Neither too fast nor too slow a growth will be desirable for producing a fermentation product presenting all the essential qualities.

The success of fermentation in breweries depends not only upon the amount of alcohol in the beer, but also in large measures upon its flavour, taste and stability. It is the aim of the brewer to conduct fermentation in such a controlled manner that by mixing a healthy type of pitching yeast with the beerwort, a rapid growth of yeast may be ensured, and the access of other harmful micro-organisms checked. It may be pointed out, however, that a small population of lactic acid bacteria (*Lactobacillus* sp.) is always present in a brew and help in the final quality of the bear. Different strains of yeast, whether mixed types or pure races, can be distinguished by their rate of fermentation or the degree to which they will attenuate under normal conditions of a particular fermentation system. The difference of attenuation* depends largely upon the flocculating properties** of yeasts. Some yeasts tend to flocculate rapidly in the wort,

* The term "attenuation" means the rate of utilisation of the food-constituents of the wort by the yeast cells.

** "Flocculating property" means the clumping power of the yeast cells either at the bottom or on the top of the fermenting liquid.

while others remain in suspension. The highly flocculating yeasts tend to attenuate the beerwort very slowly. Thus there is a reciprocal relation between flocculation and attenuation.

In breweries two methods of fermentation are adopted—(1) top fermentation, and (2) bottom fermentation. The former process is a faster one than the latter. The following experiments were, therefore, set up to ascertain the changes in the fermenting medium on inoculation with different selected strains of pitching yeast, as well as the attenuating power of these strains with a view to find out a strain of bottom fermenting yeast most suitable for the manufacture of beer under local conditions.

## Materials and Methods

Pure cultures of *S. cerevisiae*, obtained from various sources, were periodically examined, and subcultures were maintained on unhopped beerwort-agar medium. Index numbers of the yeast strains were as follows:— $M_5$, $B_1$, $B_2$, $B_3$ and $B_{10}$ .

Cooled beerwort was pumped into experimental vats (capacity 40 gallons each) and pitched with freshly-grown yeast cultures, taking care of the fact that the number of cells in the pitched wort did not exceed 3,000 cells per c.c. The vats were placed in the fermentation chamber fitted with cooling coils, and the temperature was maintained constantly at $50° \pm 2°F$.

The samples of wort from the respective vats were collected and analysed before and after pitching, according to the schedule mentioned below. (1) The specific gravity was recorded at a constant temperature of 20°C; the amount of total solids was measured in terms of °Brix at the above temperature; (2) the reducing sugar was estimated with Fehling's solutions and expressed in terms of maltose; and (3) the *p*H of the test liquor was recorded from the results of examination by B.D.H. comparator. The indicators used were Chloro-phenol Red and Bromo-cresol Green of original B.D.H. make. The results were recorded every 24 hours The wort was roused and the 'heads' were removed after the first two days of fermentation.

## Experimental Results

*Type I* ($M_5$)—This strain of *S. cerevisiae* was known to be a typical beer-fermenting yeast, imported from the United Kingdom. The growth and multiplication of the cells were neither too slow nor too fast. From Table 1, it would appear that the rate of consumption of sugar during the first 24 hours was rather faster than in the later periods of growth, when it became steady, and the process continued until the sixth day of fermentation, after which there was no further reduction of the sugar-content of the fermenting wort. Reduction of sugar, as will as fall in gravity and total solids took place, with the only exception that during the first 24 hours no rapid change of specific gravity or the reduction in the quantity of total solid was marked. The *p*H showed a very slow accumulation of organic acids in a strong biologically buffered wort. On the fourth day of fermentation the rate of formation of acids increased a little, and then no further change took place. At the end of the sixth day all the processes, e.g., consumption of sugar, change of *p*H, reduction in specific gravity and quantity of total solids stopped indicating that the primary fermentation was completed.

Table 1. *Effect of the growth of pitching yeast in beerwort during first seven days*

| Pitching yeast | Days after pitching | Sp. Gr. | Total Solid (°Brix) | Sugar (Maltose %) | *p*H |
|---|---|---|---|---|---|
| | 0 | 1.040 | 10.0 | 7.5 | 5.6 |
| | 1 | 1.039 | 9.9 | 5.2 | 5.5 |
| | 2 | 1.035 | 8.9 | 4.8 | 5.3 |
| $M_5$ | 3 | 1.030 | 7.8 | 4.2 | 5.2 |
| | 4 | 1.022 | 5.6 | 3.8 | 5.1 |
| | 5 | 1.016 | 4.2 | 2.7 | 4.7 |
| | 6 | 1.010 | 2.8 | 1.3 | 4.6 |
| | 7 | 1.010 | 2.8 | 1.3 | 4.6 |
| | 0 | 1.040 | 10.5 | 7.5 | 5.6 |
| | 1 | 1.038 | 9.7 | 5.9 | 4.8 |
| | 2 | 1.022 | 7.1 | 4.1 | 4.5 |
| $B_1$ | 3 | 1.013 | 4.7 | 2.4 | 4.3 |
| | 4 | 1.011 | 3.7 | 1.7 | 4.2 |
| | 5 | 1.010 | 3.5 | 1.4 | 4.0 |
| | 6 | 1.010 | 3.5 | 1.4 | 4.0 |
| | 7 | 1.010 | 3.5 | 1.4 | 4.0 |
| | 0 | 1.040 | 10.5 | 7.5 | 5.6 |
| | 1 | 1.036 | 9.6 | 5.3 | 5.2 |
| | 2 | 1.014 | 3.8 | 2.7 | 4.1 |
| $B_2$ | 3 | 1.010 | 3.5 | 1.3 | 4.0 |
| | 4 | 1.010 | 3.5 | 1.3 | 4.0 |
| | 5 | 1.010 | 3.5 | 1.3 | 4.0 |
| | 6 | 1.010 | 3.5 | 1.3 | 4.0 |
| | 7 | 1.010 | 3.5 | 1.3 | 4.0 |
| | 0 | 1.040 | 10.5 | 7.5 | 5.6 |
| | 1 | 1.038 | 9.7 | 5.2 | 5.2 |
| | 2 | 1.036 | 9.2 | 4.8 | 5.0 |
| $B_3$ | 3 | 1.022 | 5.8 | 3.6 | 4.5 |
| | 4 | 1.020 | 5.2 | 3.3 | 4.4 |
| | 5 | 1.018 | 4.8 | 2.7 | 4.4 |
| | 6 | 1.010 | 2.8 | 1.6 | 4.2 |
| | 7 | 1.010 | 2.8 | 1.6 | 4.2 |
| | 0 | 1.040 | 10.5 | 7.5 | 5.6 |
| | 1 | 1.040 | 10.5 | 7.5 | 5.6 |
| | 2 | 1.039 | 10.2 | 6.9 | 4.8 |
| $B_{10}$ | 3 | 1.030 | 7.8 | 5.7 | 4.4 |
| | 4 | 1.012 | 4.5 | 2.2 | 4.2 |
| | 5 | 1.010 | 3.4 | 1.4 | 4.0 |
| | 6 | 1.010 | 3.4 | 1.4 | 4.0 |
| | 7 | 1.010 | 3.4 | 1.4 | 4.0 |
| | 0 | 1.040 | 10.5 | 7.5 | 5.6 |
| | 1 | 1.036 | 9.4 | 6.1 | 5.5 |
| | 2 | 1.034 | 8.8 | 6.0 | 5.3 |
| $B_3+B_{10}$ | 3 | 1.028 | 7.4 | 5.8 | 5.1 |
| | 4 | 1.022 | 5.8 | 4.2 | 5.0 |
| | 5 | 1.016 | 4.2 | 3.1 | 4.6 |
| | 6 | 1.009 | 2.4 | 1.4 | 4.3 |
| | 7 | 1.009 | 2.4 | 1.4 | 4.3 |

*Types II and III* ($B_1$ *and* $B_2$)—These two strains of *S. cerevisiae* were obtained from a distillery, where these were used for their capability of starting vigorous fermentation in the early stage of multiplication. The sugar consumption was not too fast during the first 24 hours. The reaction became extremely vigorous on the third day of fermentation. The fall in the amount of total solid and the reduction of specific gravity followed the same course with the consumption of sugar. The increase in the acidity took place suddenly during the second day of fermentation in $B_2$, and no further change was observed until the end of fermentation. The course of reaction in $B_1$ was somewhat different from that of $B_2$. It could, however, be concluded from the experimental results presented here that these two strains possessed higher attenuating power.

*Type IV* ($B_3$)—This was a purified culture and a selected strain of *S. cerevisiae* obtained from an imported Italian wine. It appears from the experimental results that the two types $M_5$ and $B_3$, although obtained from two distinctly different sources, had nearly idential fermenting behaviour. In the case of the strain $B_3$ also the wort was left with a very small quantity of residual fermentable sugar at the end of the sixth day.

*Type V* ($B_{10}$)—This strain of yeast was obtained from a laboratory stock and purified afterwords in beerwort-agar, and was used for fermentation. The special feature of this strain was that during the first two days of incubation there was very little change in the compostion of the wort. It would appear from the table that the multiplication of yeast started after two days of fermentation, but the reaction increased suddenly. On the fourth day the process slowed down and totally stopped in course of another 24 hours. The rise in the acidity of the wort during the first 48 hour could be explained as due to the prolonged lag phase of the yeast during this period and the consequent increase in the lactic acid fermentation at the early stage.

*Type VI* ($B_3+B_{10}$)—It has been reported that if a mixture of pure cultures of two different strains of yeast is used for pitching beerwort, the behaviour of the mixed strain would show an intermediate characteristic during fermentation. In the present work the strains $B_3$ and $B_{10}$ were two extreme types as regards their capability of fermenting beerwort. With an idea of obtaining blended characters in the mixed strain dilutions of cells were made with sterile water and taken in such proportions that the mixture would contain approximately equal number of cells from each of these strains. The rate of attenuation of beerwort, however, gradually acquired the characteristics of the slow fermenting type $B_3$.

## Discussion

The strains of yeast used were of low-flocculating type and the experiments showed that the period of complete attenuation (the grade required for beer) was not more than seven days, which was taken to be suitable for beer fermentation.

Of all the yeast strains used for the experiments, $M_5$ and $B_3$ attenuated the beerwort at a uniform rate, and reactions stop at the end of the sixth day from the date of pitching. The most striking results were obtained with the strains $B_1$ and $B_2$. These cultures possessed high attenuating power, and the reactions stopped in both these cases almost after the third day of fermentation. The strain $B_{10}$, however, showed a distinctly different nature of reaction. It remained almost inactive during the first two days of fermentation (excepting the production of acids). The fermentation started at the end of the second day, and the rate went on increasing suddenly until it stopped almost at the end of the fourth day. The mixed type $B_3+B_{10}$ was used in this experiment with a view to study its behaviour towards the beerwort. Although it was expected that the resulting reaction would be a different one, yet the results showed that the characters of the slow-fermenting strain $B_3$ dominated.

There was a considerable controversy amongst the earlier workers about the superiority of a pure culture of yeast over a mixture of selected strains in the production of flavour, brilliancy and general qualities of the finished beer. The use of pure yeasts had not been adopted in several countries for top-fermentation beer. Van Laer (1894) recommended that a mixture of selected races of yeast is almost essential for a successful production of finished beer. This procedure was, however, contradicted by Jörgensen (1894), who argued that in order to introduce an element of certainty into fermenting conditions it was indispensable to use a single race of pure yeast. By a continuous trial Miller and Hyde (1894) confirmed the results obtained by Jörgensen. Brown (1934) suggested a modified system of using two or more selected races of yeast together, and his experiments proved a success after extensive trials.

It was observed in the present investigation, that single strains of pure yeast (excepting $B_1$ and $B_2$, which are found to be high alcohol-producing yeasts, and therefore, unsuitable for beer fermentation) were suitable for fermenting beerwort. After giving them a few trials, it was possible to forecast the nature of attenuation of the wort and the quality of the finished beer produced by them. The pure strain $B_{10}$, although attenuated the wort to a desirable extent, failed to produce the typical flavour. The desired flavour of the finished beer could only be obtained from brews fermented by the strains $M_5$ and $B_3$. It is interesting to note that the flavour developed in the beer by the mixed strain $B_3$ and $B_{10}$ did not correspond to that of either $B_3$ or $B_{10}$ alone. The flavour was quite different and was not at all suitable for finished beer. From the results stated above,it could be concluded that a tried single strain of pure yeast could be safely used for fermenting beerwort, while the use of mixed strains for this purpose should be considered rather risky and with uncertain effects, as the strain getting the upper hand and guiding the course of fermentation cannot be predicted without extensive trials.

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