An Occurrence of "Petite Colonie" Mutation in an Alcohol Distillery Yeast

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During the early part of March, 1956, it was noticed in one of the Colonial Sugar Refining Company's alcohol distilleries that, under the microscope, the cells of the yeast culture in use (Saccharomyces cerevisiae) were not as uniform in appearance as the cells of that strain had been in the past. The particular culture on which this observation was made was a reserve culture of the yeast which had been brought into daily use in the distillery 10 days previously.

EXPERIMENTAL METHODS AND RESULTS

Samples drawn from the different ferment vessels at the distillery and the various laboratory cultures were plated out on malt-extract agar. After incubation at 30 C for 3 to 6 days, the plates showed, in addition to normal yeast colonies, a high proportion (approximately 20 per cent) of small, but fairly uniform, colonies. At the age of 3 days the two types of colonies were approximately 3.25 mm and 1.25 mm in diameter, respectively. The small colonies retained their characteristic appearance over many cultural transfers.

The smallness of the colonies of the foreign yeast led to the suspicion that "petite colonie" mutation of the distillery yeast might have occurred (Ephrussi, 1948, 1950; Ephrussi et al., 1949; Ephrussi and Hottinger 1950).

Accordingly, the small colony yeast isolated from the

**Table 1**

<table>
<thead>
<tr>
<th>Conc of Acriflavine</th>
<th>No. of Colonies per Plate (Avg of Quadruplicate Plates)</th>
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</thead>
<tbody>
<tr>
<td>0</td>
<td>61</td>
</tr>
<tr>
<td>1:100,000</td>
<td>52</td>
</tr>
<tr>
<td>1:10,000</td>
<td>52</td>
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A microscopic examination was then made of all the other reserve laboratory cultures of the yeast which were on hand. Each culture showed the same phenomenon.

The variation in forms observed appeared to be consistent with either (1) contamination of the culture with another yeast, or (2) mutation of the distillery yeast.

**Figure 1.** Variation in colony size (incubation period of 72 hr at 30 C)
Moreover, the rates of formation of alcohol from molasses worts were found to be almost identical, although much less than that of the distillery yeast (figure 2). For this purpose, small flasks of molasses wort (28 Bx) were sown with wort cultures of the three yeasts to give the same initial concentration of cells. The rates of alcohol formation at 30°C were determined by measurement of density of distillate at regular intervals during distillation. The results obtained are shown in figure 2. It is clear from this figure that the two small colony yeasts form alcohol too slowly to be of use industrially.

The absorption bands for reduced cytochromes were examined on cells grown aerobically, harvested centrifugally and cooled in liquid air, using a Beck2 hand spectroscope. With the distillery yeast, α-bands a, b, and c were distinguishable. Both small colony yeasts showed only one distinct band, the c band.

**DISCUSSION**

In the present work we have recorded the appearance of “petite colonie” mutation in reserve cultures of a strain of yeast being used in one of our distilleries for the production of industrial alcohol from molasses.

Such an occurrence is of considerable interest to the fermentation industry. As far as we are aware, this is the first occasion on which “petite colonie” variation has been observed in cultures of yeast in industrial use.

Unfortunately, the records of the distillery involved gave us no clue to the cause of the variation, as no unusual circumstances were noted in the routine propagation of the seed yeast. In view, however, of the fact that high proportions (some 20 per cent) of “petite colonie” yeasts were observed to be present at all of the different stages of ferment preparation, it is evident that mutation must have taken place in the primary culture stage, namely, the laboratory culture.

Attention was given to the possibility that some particular batch of cane molasses used for making up worts might have been responsible for the change which occurred. Laboratory trials with the various batches of molasses in stock, however, failed to reproduce the effect.

Yet another possible explanation for the phenomenon was the production of “petite colonie” yeast through growth at an elevated temperature (Yēas, 1956) such as could occur through failure of an incubator thermostat. However, this possibility was made more remote by our failure to obtain “petite colonie” variants from the distillery yeast under the conditions described by Yēas.

Hence, the reason for the outbreak of “petite colonie” mutation in the yeast remains unknown.


A Note on the Stability of Clostridia when Held in Continuous Culture

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Most of the selected strains of microorganisms used in industry are unstable, in the sense that they undergo biochemical and morphological changes on repeated transfer to fresh medium. Especially notorious in this respect are the solvent-producing clostridia (Kutzenok and Aschner, 1952) and some cultures of Streptomyces griseus (Williams and McCoy, 1953). The early literature on degeneration of clostridia has been cited by Perlman et al. (1954) in an article describing similar behavior of an asporogenous mutant of S. griseus. The latter workers found, for example, that S. griseus lost its ability to produce streptomycin after six or eight serial transfers, although it retained its ability to use glucose and to form vitamin B₁₂. Furthermore, they confirmed the widely held view that increasing the frequency of transfer brings about more rapid degeneration.

From such reports it is often inferred that continuous fermentation is impractical, the argument being that if frequent subculture is harmful then to hold a culture in active growth all the time will surely bring about rapid deterioration. To read the literature on this point is rather confusing because some writers have carelessly used the word "continuous" to describe apparatus or procedures which were in fact intermittent. The purpose of this experiment was to find out whether continuous culture causes more rapid or less rapid degeneration than serial transfer. A solvent-producing species of Clostridium was the only test organism used, but it may be fairly typical of other unstable microorganisms. We were particularly interested in its behavior when held continuously at a low level of growth in a rich medium, that is when held in the logarithmic growth phase under conditions similar to those in the cell propagator of a multiple-tank system. The experiments of Novick and Szilard (1950) with the "chemostat" are not comparable because growth was limited by a scarce nutrient rather than by washout of the cells.

MATERIALS AND METHODS

All of the work was done with a strain of Clostridium saccharoacetobutylicum which has been described in the patent literature (Woodruff et al., 1937). Solvent production in a molasses medium is normally about 30 per cent of the sugar fermented, and composition of the solvent is roughly 70 per cent butanol, 26.5 per cent acetone, and 2.5 per cent ethanol. The medium used here was similar to that of Kutzenok and Aschner (1952) and consisted of 0.5 per cent peptone (Difco),

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