

# THE DISCRIMINATION OF THE VARIOUS SPECIES OF SACCHAROMYCETES.

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THIS has been considered hitherto simply a matter of microscopic observation ; Rees and others have given descriptions and figures of the various yeast plants, but it has been recently shown by Hansen that each species of yeast is capable of assuming, under varying conditions of cultivation, etc., nearly all the forms which had been assumed as properly belonging to the others. Thus a circular, or nearly circular form is associated with the *Saccharomyces cerevisiæ* group, an oval one with the *Ellipsoideus* group, and an elongated or sausage-shape form with the *Pastorian* group ; but as under certain circumstances *Sacc. Cerevisiæ* forms oval and spindle-shaped cells, and the others in their turn appear as circular cells, any absolute determination of their species by the microscope alone must naturally be futile. As will be seen further on, the ability to differentiate the various species of yeast is likely to become a matter of considerable importance, and one which the analyst ought to be prepared to undertake. Before going into the question of resolving yeast into its various species, it will be well to say a few words about yeast itself.

The true yeasts are simple unicellular plants belonging to the genus *Fungi*, and as they are capable of developing spores in cells called *asci*, they belong to the *Ascogenous* division of that genus. Yeast can reproduce itself under two distinctly different conditions ; the ordinary every-day one, where, immersed in a liquid capable of undergoing fermentation, it rapidly increases by a process of budding, never in this case forming spores. The other condition was first brought into notice by Rees in 1869, who found that by suddenly depriving yeast of all saccharine food, and placing it on the surface of a slice of potato or other moist porous vegetable root, in a very thin layer, budding still went on for a short time, but eventually ceased, and in a number of the cells spores were formed. Other observers obtained the same results by cultivating yeast on blocks of plaster of Paris, kept moist ; and this more convenient method is the one now universally employed for this purpose.

To Pasteur we owe our first definite knowledge of the relation of the organised ferments to the process of fermentation. He conclusively proved that fermentation only proceeds when the ferment grows and multiplies ; he also taught us that such organised ferments as the acetic, lactic, butyric, etc., together with *baccilli*, bacteria, etc., frequently contaminated our brewing yeast, and were the causes of what are commonly known now as the diseases of beer, viz., acidity, instability, ropiness, etc. He also very clearly pointed out the way to get rid of these troublesome invaders, and in this way did inestimable service to the brewing industry. He, however, states that when you have thoroughly purified a yeast, so as to be entirely rid of all adventitious organisms, you may yet obtain a yeast which gives a beer of bad flavour. This fact, which Pasteur left unexplained, has been entirely cleared up by the brilliant work of Dr. Hansen, the Principal of the Physiological Laboratory at Carlsberg, Copenhagen. He found that yeast, as ordinarily used in breweries, is an indefinite mixture of yeast plants of various species and varieties ; that the species and variety exercise a most marked influence on

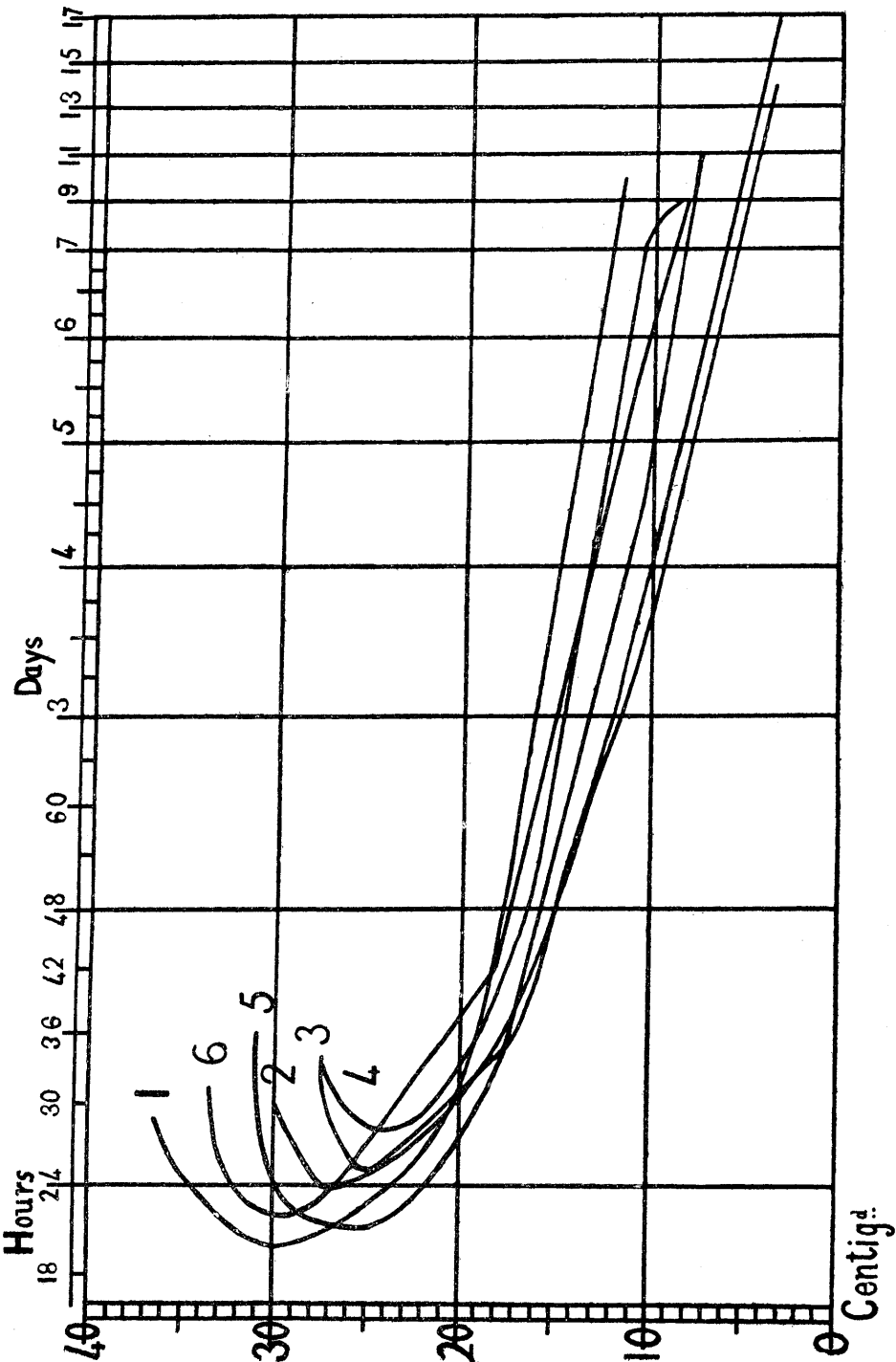
the flavour and properties of the finished beer, some causing a nauseous, bitter flavour others producing an article which will not clarify, others an unstable article, etc. He proposed to introduce into practice, and has actually done so, yeasts grown from a single cell, and therefore consisting of one distinct species only. The advantages of this system are obvious; the brewer, provided he takes care to use good materials, can invariably secure a beer of identical flavour and other properties. That this is not a mere matter of laboratory experiment is shown conclusively by the fact that these single cell yeasts have been used since the year 1884 in the Old and New Carlsberg Breweries, which produce annually over seven million gallons of lager and export beer, with the most marked success. In Denmark and Norway they are successfully used in all the largest breweries, to some extent in Sweden, Finland, Bohemia, and other parts of Austria, in Switzerland, North Italy, Belgium, North America, Russia, Germany, and Holland, in Asia and Australia. In all these breweries, with the exception of Baartz and Zoon, at Rotterdam, the system of low fermentation is adopted, *i.e.*, the type of yeast used is a bottom fermentation one, the fermentation being conducted at a temperature of 6 to 10° Cent.; the yeast sinking to the bottom of the fermenting vessel. The Rotterdam brewery employs the high fermentation system, which is the one almost, if not invariably, used in this country. In this system a yeast is employed which, as fermentation proceeds, rises to the surface, the temperature of the fermentation being from 10 to 15° Cent. So far the high or top fermentation yeasts have been very little studied, but as the advantages to be obtained by employing single-cell yeasts are sure to be recognised sooner or later in this country, a highly-interesting field of practical observation and research is opened out. The preparation of the small samples of the different varieties of single cell yeast will naturally fall within the province of the chemist; their multiplication to quantities sufficient for practical purposes, will take place in properly constructed apparatuses in the brewery.

The method devised at first by Hansen to obtain a sample from one cell was as follows: he diluted the yeast with water so that a measured quantity under the field of the microscope contained, say twenty cells. A quantity of this mixture, equal to that contained in the microscope field, was added to 20 c.c. of water and well shaken up. It was now assumed that one c.c. of this mixture would contain a single yeast cell. This quantity was next added to wort contained in a Pasteur or similar flask, and vigorously shaken, the remaining 19 c.c. being treated in a similar manner. The flasks were examined in a few days, and if bubbles were seen proceeding from one point only, the experiment was considered successful. If no sign of fermentation appeared, it was evidence that the c.c. of the mixture introduced had not contained a yeast cell; if bubbles arose from two or more points, that more than one yeast cell had been introduced, and naturally such flasks were rejected. This method had considerable elements of chance about it, and I only mention it as the one by which all Dr. Hansen's discoveries were made, particularly as it has been hinted by some of his detractors that his studies had their origin in Koch's gelatine plate cultivation process. This could not be the case, because all Dr. Hansen's facts were published before the latter's gelatine process had been announced.

After the publication of Koch's method, Hansen adopted a modification of it,

which leaves nothing to be desired in the way of obtaining cultures from a single yeast cell. He proceeds as follows: A sample of yeast is diluted as before with water until each microscopic field shows about twenty or thirty cells; a small drop of this mixture is now mixed thoroughly with 20 c.c. of ordinary beer wort containing 10 per cent. of gelatine. In all these processes it is almost unnecessary to state to my present audience that apparatus and fluids used must all be thoroughly sterilised. The gelatinized wort must never be allowed to reach a higher temperature than 25° Cent. at the time of the addition of the yeast cells or afterwards, for obvious reasons. A drop of this is spread in an even layer on an ordinary microscopic cover glass, placed film side downwards, on a small moisture chamber, containing a small quantity of water. The whole is placed under the microscope, and if the experiment has been successful, some half-dozen isolated yeast cells will be observed in different parts of the field, each of which is destined to form a colony. Those sufficiently apart are chosen from the rest, and a ring marked round them by a small apparatus which screws into the place of the object glass. A number is affixed to each circle with a fine pen, and the figure of each cell and its number noted down in the memorandum book. The moist chamber and its contents are placed in a warm place for 24 hours and further examined. Each cell will now be found to have formed a small colony; all the marked colonies are carefully examined, to see they are not likely to coalesce with adjacent ones, and that they are perfectly circular, great stress being laid upon this latter point by Dr. Hansen. After seeing that all is going on right, the moist chamber is allowed to stay another 48 or 72 hours, when the colonies will be found so large as to be easily seen by the naked eye. They are each in turn picked off the gelatine film on small pieces of platinum wire, which are dropped, colony and wire, into separate cultivation flasks containing wort. Fermentation and multiplication of yeast cells now commences, and all that is necessary is the careful periodical changing of the wort, so as to avoid outside contamination. By this means it is possible to increase the growth of yeast to any amount. The next question was to establish a method of differentiating the species with certainty, and this problem our indefatigable observer, Dr. Hansen likewise solved satisfactorily. By observing the occurrence of spore formation at different temperatures, he discovered that each species only developed spores between certain temperatures, and that at temperatures between these limits each species had its own relation to time and temperature. By taking the times of development as abscissæ, and the temperatures as ordinates, a curve may be generated for each yeast.

The accompanying diagram represents such a series of curves for six yeasts. For the abscissæ I have used a logarithmic scale of numbers, by which the initial curves are seen more plainly than if the ordinary numerical scale had been used. Spore formation is best observed in the following manner: A small truncated cone of plaster of Paris is moulded, the upper surface of which must be perfectly flat and smooth. On this surface, the yeast to be observed must be spread in a thin layer, the cone is placed base downwards in a vessel, and water poured in until it reaches halfway up the side of the cone. The whole is loosely covered, so as to admit of free aeration, this being absolutely necessary for spore formation, and kept at an even temperature. Small samples are taken off the surface of the cone from time to time and examined under the micro-



scope; the time to be noted is the earliest sign of spore formation. It has been found that only young and vigorous cells sporulate; care must be taken, therefore, to secure these by growing the sample for 24 hours in good, well-aerated wort, carefully pouring this off, and adding a fresh supply of wort. After another 24 hours the wort may be carefully poured off the thick layer of yeast at the bottom of the vessel, and the latter spread on the plaster block as described above. In this manner Hansen has differentiated six species of yeast, *Saccharomycetes Cerevisiæ*, a top fermentation yeast, having its times of spore formation as follows:—

Sacc. <i>Cerevisiæ</i> I.				
37·5	...	...	...	No spore formation.
36·37	...	...	...	29 hours.
35	...	...	...	25 "
33·5	...	...	...	23 "
30	...	...	...	20 "
25	...	...	...	23 "
23	...	...	...	27 "
17·5	...	...	...	50 "
16 5	...	...	...	65 "
11-12	...	...	...	10 days.
9	...	...	...	No development.

*S. Pastorianus* I., a bottom fermentation yeast, which frequently occurs in the air of fermenting rooms, and gives a strong, bitter taste to beer.

*S. Pastorianus* II., feeble top fermentation yeast, does not seem to give rise to any disease in beer.

*S. Pastorianus* III., top fermentation yeast, causes yeast turbidity.

*S. Ellipsoideus* I., bottom fermentation yeast, found on the surface of grapes.

*S. Ellipsoideus* II., bottom fermentation yeast, causes great turbidity.

As the whole nomenclature of yeasts is at present in a state of transition, these names must be looked upon as merely provisional.

In addition to the before-mentioned discoveries in spore formation, Dr. Hansen observed some curious facts with reference to the formation of yeast films on the surface of a yeast cultivation when fermentation had quite ceased. Each yeast seems capable of forming a film, which appears at a longer or shorter period of time for each species. Temperature effects a similar acceleration or retardation, as in the case of spore formation, and consequently the conditions of film formation may be used to corroborate those of spore formation.

From the foregoing we conclude that diseases of beer are not only caused by false ferments, but also by *saccharomycetes*; that it is possible to distinguish the useful from the noxious species of the latter; and that it is easy to prepare a yeast which shall consist of one species only, thus affording the brewer an exactitude and certainty in his fermentation results he never possessed before, and at the same time opening out a wide and interesting field for the analyst.

#### DISCUSSION.

The PRESIDENT said he would like to know if Dr. Sykes could explain definitely the cause which determined the matter as to whether a yeast became a top or a bottom

yeast. Was it due to an entirely different kind of cell, or was it due to the circumstances under which the cell was placed? Had Hansen's researches confirmed the belief a yeast became top yeast, when by its treatment the growth was encouraged and became vigorous, or on the other hand bottom yeast when its growth was checked. Of course it was understood that top yeast fermentation took place at a higher temperature, and that in proportion the growth of the yeast plant was more rapid, and thus gas was formed more abundantly, which, by adhering to the yeast cells, rendered them buoyant and carried them to the top, so forming top yeast; but with the lower temperature and less vigorous growth and less gas formation, bottom yeast resulted.

With respect to the modification which Hansen has shown by his experiments to be capable of cultivation, is it not true that these varieties are very unstable, and readily revert to the ordinary type?

He, the speaker, would like also to ask Dr. Sykes whether he had any knowledge of the enduring qualities of the ascospore formation, as compared with the ordinary method of reproduction. From a biological as well as a sanitary point of view, the matter was of great interest to him.

Mr. RICHMOND, bearing in mind that Dr. Sykes had stated that *Sach. Pasteureanis I.* gave bitter taste to beer, would like to know if the substance giving this taste was precipitated by acetate of lead, otherwise it might interfere with the detection of foreign bitters.

Mr. CASSAL asked if they were to understand that one sort was absolutely incapable of being converted into any other sort? He would like to know if any experiments had been made with a cell as to its capability of altering its original constitution as evidenced, for example, by the appearance of abnormal products, such as the bitter substance which had been alluded to. It had been asserted in regard to micro-organisms obtained from "pure cultivations," that they were incapable of change in this respect, but he greatly doubted whether there was sufficient evidence to warrant such a statement.

In reply to the President's questions Dr. Sykes said the attempt to transform one species of yeast into another had been tried in every conceivable way by Hansen, and found impossible; he considers that the species are as distinct and definite as that of any of the higher fungi. You may cultivate high yeast at a low temperature, but though it sinks like a low yeast, fermentation proceeds much more slowly than in the case of a bottom fermentation yeast; if placed in its normal fermentation temperature, and especially if well aerated, it quickly resumes its former properties. In making a gelatine cultivation of a yeast known to be of one species, you might find two colonies of cells of entirely different shape, as, for instance, one oval, the other sausage-shaped. If these were introduced into two flasks, and a cultivation started, though the whole cells formed at first would be of the same shape as their progenitors, yet sooner or later this speciality of form would entirely disappear. With reference to the spores of yeast they are remarkably persistent. About a year ago he had found that they existed in large quantities on malt, which is generally exposed to a temperature of 200° to 220° F. for several hours during the final stage of drying on the kiln.

In reply to Mr. Richmond's question, he did not know whether the bitter generated by *S. Pastoriensis I.* was precipitable by acetate of lead or not.

In reply to Mr. Cassal, Dr. Sykes saw that whatever changes took place in the shape, etc., of the cells by different methods of cultivation, their chemical properties remain unaltered; a *Cerevisiæ* would always produce a good flavoured beer, a *Pastoriensis I.* would invariably give one of bad flavour.

*(Conclusion of the Society's Proceedings.)*