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On the Longevity of certain Species of Yeast.

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In the autumn of 1918 Dr. J. J. Hood handed to one of us (A. R. L.) eight cultures of yeast, belonging to the late Mr. A. Gordon Salamon. The cultures in question, it appears, were given to Mr. Salamon by the late Prof. Hansen in 1887. They consisted of Freudenreich flasks, containing wads of perfectly dry cotton-wool. The flasks were furnished with a side tube and there was a tube at the apex of each hollow stopper. There was a cotton-wool plug at the opening of each flask inside the hollow stopper, and the tube at the apex of the stopper was plugged with cotton-wool, as was also the side tube. In addition to this, the cotton-wool plug of the side tube was coated with sealing-wax and the hollow stopper was also fixed on with a ring of sealing-wax. Each flask bore a label, giving the name of the particular yeast and

the date in Prof. Hansen's handwriting. The following is a copy of the script on each label :—

Sacch. Pastorianus I, Hansen, 17/9/87.
Sacch. Pastorianus II, Hansen, 17/9/87.
Sacch. Pastorianus III, Hansen, 17/9/87
Sacch. ellipsoideus I, Hansen, 17/9/87.
Sacch. ellipsoideus II, Hansen, 17/9/87.
Sacch. exiguus, 17/9/87.
Sacch. cerevisiæ I, Hansen, 17/9/87.
Carlsberg Unterhefe, No. 2. 17/9/87.

As already observed, the contents of each flask consisted of a dry cotton-wool plug and the plugs were in every case of a brownish colour. Apparently, the cultures had originally been made in the manner described by Prof. Hansen in his 'Studies in Fermentation,' 1896, p. 74, thus :—

"For sending pure cultivated yeast in an absolutely pure condition, and in such a manner that its multiplication can be effected easily and with certainty, I have likewise made use of the following method, which has also given good results :—To the small cylindrical flasks generally known as Freudenreich flasks I have added a side tube (see fig.). The tube, A, on the hood is, as usual, filled with cotton-wool; a firm layer of cotton-wool is placed at the bottom, E, of the flask, and a plug is inserted in the neck, B. The side tube is also plugged with cotton-wool, and the flask is then sterilised by heating it for two hours at 150° C. When it has cooled, the tube is joined to the rubber of a two-necked flask, in which the yeast has been grown, and a drop of the fairly thick yeast is poured on to the layer of cotton-wool, E. The tube is then closed by a stopper, D, of asbestos card, previously sterilised in a flame, and the stopper is then coated over with a layer of sealing-wax, C."

In order to ascertain if the yeasts were still alive, a little sterile sweet wort was introduced into each flask by the side tube after removing the wax and cotton-wool. The flasks were then kept in an incubator at 25° until growth appeared. A thick sediment formed after some time, and when this was examined it was found in every case to contain yeasts, sometimes contaminated with bacteria. Plate cultures were made on wort gelatin containing 0·4 per cent. of tartaric acid, and ultimately pure cultures of the several yeasts were obtained.

The cultures were identified by Hansen's spore method, the results being shown in the following Table :—

Hansen's Yeasts.

Species.	Time of first appearance of spores observed in a fresh 24 hours' growth at 25° C.	Size of the spores.
<i>Sacch. cerevisiæ</i>	Within 24 hours	2½-6μ
<i>Sacch. ellipsoideus</i> I	Within 24 hours	2 -4μ
<i>Sacch. ellipsoideus</i> II	Within 36 hours	2 -4μ
<i>Sacch. Pastorianus</i> I	Within 36 hours	1½-4μ
<i>Sacch. Pastorianus</i> II	Within 24 hours	2 -4μ
<i>Sacch. Pastorianus</i> III	Within 36 hours	2 -3½μ
<i>Sacch. exiguus</i>	Scanty spores after 36 hours	2 -4½μ
Carlsberg No. 2	Within 36 hours	3 -6μ

We may mention that four species of these yeasts showed a well-marked liquefying power towards gelatin, liquefaction occurring in from 2½ to 4 weeks after making the streaks and keeping at the ordinary temperature. The liquefying power occurred in the order shown below:—

1. *Sacch. Pastorianus* I.
2. *Sacch. ellipsoideus* I.
3. *Sacch. cerevisiæ*.
4. *Sacch. Carlsberg*, No. 2.

The other species did not liquefy gelatin, but here it was observed that there was a characteristic tendency for growth to extend into the depth of the gelatin culture medium.

The fact that after thirty-four years these yeasts were still living is, we think, a point of great scientific interest and importance. In what form they retained their vitality, however—whether as resting cells or spores—cannot, unfortunately, be determined. From the work of Hansen we know that one species at least, *S. apiculatus*, found on the exterior of certain fruits, hibernates in the soil, and inasmuch as this species, or at all events the yeast with which Hansen worked, does not form endo-spores, it may be that our yeasts have been preserved as resting cells.

The only observations at all parallel with our own are those of H. Will,* who showed that certain wild yeasts, on a dry asbestos medium, were still living after seventeen years. They apparently had retained the cell form.

Further experiments with these and other species of yeasts are in progress in these laboratories.

* 'Zeitschrift für das gesammte Brauwesen,' 1896-1904.