LXX. STUDIES ON THE GROWTH OF YEAST.

II. A FURTHER NOTE ON ALLELOCATALYSIS.

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IN a recent communication Robertson [1924] has claimed that the failure of Cutler and Crump [1923] to observe allelocatalysis in cultures of *Colpidium colpoda* was due to the omission of the precaution of washing the organisms before inoculation. In my previous communication on the subject [1924] I failed to observe allelocatalysis in cultures of yeast, but I also had omitted to wash the yeast cells except in two of the experiments. In these two experiments, as stated at the time, some doubt existed as to whether the washing process had not actually added some organic matter to the culture owing to the technique employed. I have therefore repeated two typical experiments with the improved technique described in the preceding communication. Before suspending the cells in the required medium for the drop cultures, I washed them in the following manner.

METHOD.

The apparatus consisted of a large boiling tube which was plugged tightly with cotton wool at its open end. The plug was covered with fine-meshed muslin to prevent pieces of it from becoming detached and drawn down into the medium during the centrifugalisation. The edges of the muslin were firmly attached to the rim of the tube to keep the plug in position. Through the plug and muslin passed a Dreyer's pipette also plugged at its wider end. In the boiling tube were placed five small test tubes of narrow bore, separated from the bottom of the tube by a small pad of cotton wool. These tubes bore permanent identification marks so that they could be readily distinguished from each other. Before filling them with medium the whole apparatus was thoroughly cleansed and sterilised. From 2.0 to 2.5 cc. of the sterile synthetic medium (Medium 2) were then placed in each of the tubes under aseptic conditions, and the whole was again sterilised by steam heat in the Koch steriliser for 45 minutes on three successive days.

About 0.25 cc. of the culture containing the cells to be washed was admitted to the apparatus by a capillary pipette inserted into the Dreyer

pipette. The plug was replaced with the usual precautions and a rubber teat was used to force the fluid containing the cells down into the first tube of synthetic medium, which was then gently agitated by passing a few bubbles of air through it. After standing a few minutes the whole apparatus was placed in a large centrifuge. The cells, which in the case of the yeast used sink very readily, were brought to the bottom by 30 to 45 seconds' centrifugalisation at about 600 r.p.m. The supernatant fluid was now pipetted off and a fresh amount of medium introduced from the next tube. The washing and centrifuging were then repeated.

It was found that the cells could be very thoroughly washed by this method with only the slightest risk of infection, provided that the apparatus was so arranged and care was taken to see that the Dreyer pipette was only moved in the wool plug as little as possible, in both horizontal and vertical directions. Moreover, the mortality among the washed cells was 50 % in Medium 1 and 28 % in Medium 2—figures which compare favourably with those for the previous experiments in which the cells were not washed.

RESULTS.

By the technique described above, the cells used in these experiments were washed five times with from 2.0–2.5 cc. of synthetic medium. Assuming that one-tenth of a cc. of medium was left after each washing, then the concentration of any extraneous matter introduced with the cells in the first instance was reduced after washing five times to less than $1/3,000,000$. At the end of the washing process we can assume, therefore, that we are dealing with yeast cells in synthetic medium free from any impurities other than those which may be present in the medium itself owing to the difficulty of preparing absolutely pure chemicals.

After washing, some of the cells were suspended in synthetic medium (Medium 2), others in the medium containing commercial cane sugar (Medium 1). The results are given in the following table in which is recorded the number of cells present after a given time in cultures which contained originally one, two or three cells. The cultures were grown at 25° and observations taken as described in Part I.

Exp. 10, Medium 1. Growth after 34 hours at 25°.

1 cell	2 cells
10, 8	9, 12, 9, 19

The corresponding drop volumes were, in mm.³,

103, 23	23, 16, 16, 16
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Exp. 11. Medium 2. Growth after 45 hours at 25°.

1 cell	2 cells	3 cells
3, 5, 4, 2	10, 7, 8, 3, 5, 4, 5	9, 6

The corresponding drop volumes were, in mm.³,

106, 23, 106, 19	10, 17, 23, 19, 17, 17, 51	31, 12
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No cultures in these experiments have been excluded from the table on account of exceptionally high growth, and all were examined after fixing and staining and found to be free from bacteria. The absence of any effect which could be termed allelocatalytic was confirmed at all the other periods at which the cultures were examined, as well as at the times recorded in the table. The results show that allelocatalysis does not occur in the case of the yeast studied even after washing the cells before inoculation.

CONCLUSION.

The failure to observe allelocatalysis in the experiments recorded previously was not due to the omission of the precaution of washing the cells before inoculation. My previous conclusion was therefore justified, namely, that allelocatalysis does not occur in the case of the yeast studied.

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