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Microbiology. — *"Penetration of methyleneblue into living cells after desiccation"*. By Prof. Dr. M. W. BEIJERINCK.

(Communicated in the meeting of December 28, 1912).

It is generally known that methyleneblue does not enter living yeastcells, when these are first soaked with water or swimming in a fermenting liquid, whilst it colours the dead cells intensely. It is even possible several days to cultivate yeast in wort, coloured dark blue with this pigment, without the cells being coloured in the least. On wortagar plates with methyleneblue, colourless colonies will develop. On these facts a method is based to ascertain in living yeast the number of dead cells, which gives very good results.

Meanwhile there is an exception to the rule that the cells, colouring blue are dead, and this exception will be more closely considered here.

At the examination of dried yeast, most cells of which take a dark blue colour with methyleneblue, whilst only a very small percentage remain colourless, the fermenting power often proves so great, that no other explanation can be given, but that the blue-colouring cells have for the greater part preserved that power. This is not unexpected, for it is well known that the alcoholic function is more permanent in dieing cells than the power of growth. Meanwhile, counting-experiments, whereby on one hand the number of cells colouring with methyleneblue was microscopically determined, on the other hand by plate culture, that of the cells growing out to colonies, showed that from certain dry yeast samples a much greater percentage of colonies developed, than the percentage of cells not colouring with methyleneblue. This fact was indeed unexpected and induced to a more minute observation.

First of all it was proved that the number of cells, colouring in a dilute solution of methyleneblue, depends on the way in which the solution is brought into contact with the cells. If this is done by introducing dry yeast into the solution, all the cells colour dark-blue and cannot be distinguished from the dead ones. In plate cultures, however, a greater or smaller number of colonies may be obtained from these cells, although all seem perfectly alike in their dark blue colour, and should be considered as dead by anyone ignorant of their origin. In favourable circumstances the number of colonies mounts even to 100 %, which is to say, that all the cells may colour blue and still grow out to colonies.

This is in particular obvious when the cells are beforehand coloured with methyleneblue, and the coloured material is used for

sowing; it is easy then to recognise the blue cells on the plate and watch their germination under the microscope. The blue colour is then commonly seen to disappear before the formation of buds begins. But many of the later germinating cells remain blue and produce colourless daughter-cells. I never saw young cells taking the least trace of blue from the mother-cell.

But if the dried cells are beforehand allowed to swell up in wort or in water and if the soaked material is laid in the methyleneblue solution, which is the usual way to effect the colour reaction, the result is quite different. Then only part of the cells assume the colour and this part is the smaller as the cells have longer remained in the uncoloured solution. A certain percentage, however, continue to take up the colour without having lost their reproductive power, and it seems to be very difficult to soak these cells with water.

The simplest way to effect these experiments is by using dry yeast, quite free, or nearly so from dead cells. I obtained it by centrifugation of the small-celled variety of pressed yeast from strong fermentations, these being in their most active state.

To this end it was cultivated at 28° C. in nearly neutral wort, after 6 to 8 hours brought into the centrifuge, and then quickly transferred to filterpaper in a thin layer for desiccation.

The large-celled variety of pressed yeast is less resistant to drying. To compare the two varieties, of which the smallcelled is richer in protoplasm than the other, the yeast must very cautiously be dried, first at low temperature, e.g. 25° C., then at a higher one, e.g. 50° C. This precaution is not, however, necessary to render the blue-colouring of the dry living cells visible; to this end drying of common yeast at room temperature will do.

I have, however, also met with commercial dry yeast satisfying the requirement of containing hardly any dead cells at all, namely the "Konservierte Getreide Brennerei Hefe" of the yeast works of HELBING in Hamburg, which was sent directly from the manufactory. This preparation is delivered in solidly closed tins, but after some time it loses its power of growth and fermentation; its quality thus evidently depends on the length of time past since its fabrication. It seems that this loss corresponds to that of the germinative power of seeds, which depends on their state of humidity. I possess some more preparations from the same factory, that have hardly any fermentative power and contain no cells fit for reproduction, but they have not been directly got from the manufactory and are already some years old.

When using seed of *Brassica rapa*, soaked in solutions of 1 per