CXIV. ALLELOCATALYSIS AND THE GROWTH OF YEAST.

BY GEOFFREY LEWIS PESKETT.

From the Biochemical Department, Oxford.

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BRAILSFORD ROBERTSON [1921] has claimed that in the case of certain infusoria, if two individuals are isolated into a drop of culture medium the growth produced is in a given time far more than double that produced by a single cell under similar conditions. He claims further that this phenomenon, to which he has given the name "allelocatalysis," only occurs provided that the organism is supplied with a substance, termed by him X, which is produced in media as a result of bacterial metabolism and accelerates the growth of single cell cultures of the organism. His conclusions have been based on experiments in which isolated *Enchelys* have been grown in media containing bacteria, and I can find no records in his experiments of any bacteria-free cultures. Cutler and Crump [1923], working with a culture of *Colpidium* which contained only one contaminating organism, were unable to corroborate Robertson's claims.

In the following communication I have recorded the results of some experiments in which a pure culture of yeast has been grown free from bacterial contamination in hanging-drop preparations containing one, two, three, and in a few cases five cells. The growth has, moreover, been studied in media containing various concentrations of "bios." The conditions therefore have been strictly analogous to those under which Robertson has observed the occurrence of allelocatalysis, except that the bacterial factor has been eliminated. Experiments are at present in progress to determine how far the growth of yeast may be influenced by the presence of bacteria.

EXPERIMENTAL.

General procedure.

For convenience a basal medium was prepared which had the following composition:

Basal Medium.

Primary potassium phosphate (KH_2PO_4)	5.00 g.
Ammonium chloride	2.50 g.
Magnesium sulphate	0.35 g.
Calcium chloride	0.25 g.
Glass-distilled water to 1000 cc.	

The media used in these experiments were prepared from the basal medium as follows:

Medium 1. Basal medium + 50 g. per litre commercial cane sugar.

Medium 2. Basal medium + 50 g. per litre recrystallised cane sugar.

Medium 3. Basal medium + 50 g. per litre recrystallised cane sugar, to which was added one quarter of its volume of a solution containing 12.0 mg. per 100 cc. of a solid "bios complex."

The recrystallised cane sugar was prepared from a commercial sample by three crystallisations from hot 80 % alcohol, according to the method of Willaman and Olsen [1923].

The "bios complex" was prepared according to the method of Eddy, Kerr and Williams to whom I am indebted for their courtesy in sending me details of the method previous to its publication.

Unless otherwise stated distilled water was obtained by distilling tap-water from alkaline permanganate in an all-glass apparatus, and collecting the middle portion of distillate. The first portion was used for rinsing glassware, etc., previous to sterilisation.

The salts used were Kahlbaum's "Zur Analyse" and with the exception of the calcium chloride were recrystallised twice from the glass-distilled water.

All glassware was stood for at least twelve hours under a mixture of nitric and chromic acids, rinsed first in running tap-water, then ordinary distilled and finally in glass-distilled water. It was then dried at 110° before use, with the exception of cover slips, which were dried by rinsing in 70 % alcohol (containing 0.5 % HCl) and wiping with a clean dry cloth. As far as possible the glassware used was Jena or Pyrex. All glassware was sterilised by dry heat at 170°–180° for twenty minutes. For sterilisation cover slips were put up in deep Petrie dishes (3 inches by 1½ inches), and depression slides in plugged boiling tubes. The media were sterilised immediately they were made up by steam heat in a Koch steriliser on three successive days. The vaseline used throughout these experiments was sterilised by heating to 170°–180° for twenty minutes. Silica pipettes, which could be sterilised by heating to redness in a flame, were found useful for many of the operations [Peters, 1921].

The culture used was a pure culture of *Saccharomyces cerevisiae* obtained from the Lister Institute (Nat. Type Culture 381/21). The stock culture was grown in 5 cc. portions of Medium 1 unless otherwise stated and repeatedly subcultured (about every three days). All operations involving the exposure of sterile cultures were conducted with the usual precautions against bacterial contamination in a cupboard fitted with glass back and front, which was steamed for about half an hour on the day before use, and remained closed until the time of experiment.

Method.

The procedure in each experiment was as follows. It is based on the method used by R. J. Williams [1919]. From the culture selected for growth a

sample was removed, taking care to obtain as many yeast cells as possible from the bottom of the tube in a minimum volume of medium. This sample was immediately placed in a test-tube, and may be called, for convenience, the "parent culture." Having placed in readiness a depression slide ringed with vaseline, inverted and held in a clip over a cover slip, the "parent culture" was shaken thoroughly and a small sample removed in a capillary pipette. This sample was then immediately placed out in drops on the cover slip, and the depression slide brought down carefully so as to pick up the cover slip without touching the cultures with vaseline. The slide was then inverted and the edges of the cover slip gently pressed down to make an air-tight ring of vaseline. In the later experiments a platinum nib, mounted in a glass rod, was found more convenient than a capillary pipette for placing out the culture in drops.

By a count of the average number of cells in these drops of "parent culture," and by appropriate dilution of another sample of the "parent" with the required medium, a tube of culture was made up from which a series of hanging drop preparations were made containing the required number of cells per drop. To diminish the chances of contamination it was usual to make up a fresh tube of culture for every four or five slides.

It was usual to put up at the same time one slide containing drops of medium which had been lightly infected from a very mixed bacterial culture. This was grown as a control at 25° to show the degree of bacterial growth to be expected in any accidentally infected culture, and to make sure that the technique of drying and staining the cultures was adequate to show up bacteria.

After a little practice it was found that the maximum time during which the cover slip was exposed did not exceed thirty seconds. All the operations were, moreover, conducted in the aseptic cupboard as far away from the sterilising burner as possible. By these means the risk of infection or evaporation of the cultures was minimised, and it was actually found that only a very small proportion of the cultures had to be discarded on account of the presence of bacteria.

Each slide was examined under the microscope immediately after it was put up, and the position of each drop which contained cells was recorded, together with the number of cells it contained. Buds of less than half the diameter of an average sized yeast cell were not counted, those of which the diameter was more than half that of an average sized cell were reckoned as cells. The slide was then placed in the thermostat at 25° and the time noted. At various definite intervals after the time of incubation the slides were examined and growth recorded, and during the course of the second day of growth the cultures were examined under the high power (magnification $\times 700$) and the absence of bacteria confirmed by observation.

As an approximate estimate of the drop volumes the diameter of the drops was measured and recorded. Most of the cultures in each experiment were in

drops of the same size. In a few cases, however, there were marked differences in size, but in none of these did there appear to be any marked changes in the growth. Experiments are in progress to determine whether there is any influence of drop volume on the growth of drop cultures, as the subject is of some importance in connection with the problem of "bios" and Pasteur's original observation.

At the conclusion of the experiment each cover slip was treated as follows. The position of the cultures was marked temporarily by means of Indian ink. The cover slip was carefully removed from its slide and permanent marks etched on it by means of hydrofluoric acid to establish the identity of each culture. The drops were dried off *in situ* over phosphoric anhydride *in vacuo* at 40°. The cover slips were now treated as bacteriological smears, i.e. they were fixed, stained by the Claudius' modification of Gram's technique, and mounted for bacteriological examination under an oil immersion objective. By this means it was possible to establish with certainty the presence or absence of bacteria.

RESULTS.

In Table I, I have recorded the results obtained in four experiments. The only cultures in these experiments which have been omitted from the table are three in which the growth was so extremely slow that it had not commenced until after the thirtieth hour. All the cultures were free from bacterial contamination according to the tests described above, and no culture has been omitted on account of exceptionally high growth.

Table I.

<i>Exp. 1. Medium 2</i>				
Number of cells present after 40 hours at 25° in cultures containing originally				
1 cell	2 cells (A)	2 cells (B)	3 cells (B)	5 cells (B)
		1 growing?	1 growing?	2 growing?
9, 17, 16, 11, 16, 19, 18 (15.1)	22, 32, 28 (27.3)	8, 8, 14, 9 (9.8)	13	32
The corresponding drop diameters were, in millimetres				
0.9, 0.8, 0.9, 0.6, 1.0, 0.9, 0.8 (0.84)	0.9, 1.0, 1.0 (0.97)	0.7, 0.7, 1.0, 0.8 (0.80)	0.8	1.0
<i>Exp. 2. Medium 1</i>				
Number of cells present after 32 hours at 25° in cultures containing originally				
1 cell	2 cells (A)	3 cells (B)	4 cells (B)	
		2 growing?	2 growing?	
4, 5	7, 8	7	6, 5, 8 (6.3)	
The corresponding drop diameters were, in millimetres				
0.4, 0.5	0.6, 0.4	0.4	1.0, 0.6, 0.5	
<i>Exp. 3. Medium 1</i>				
Number of cells present after 18 hours at 25° in cultures containing originally				
1 cell	2 cells (A)	3 cells (A)	5 cells (A)	3 cells (B)
				1 growing?
28, 30, 27 (28.3)	70, 89	95	125	31, 37, 27, 45 (35.0)
The drop diameters were not measured.				

Exp. 4. Medium 3

Number of cells present after 24 hours at 25° in cultures containing originally				
1 cell	2 cells (A)	3 cells (A)	2 cells (B) 1 growing?	3 cells (B) 2 growing?
7, 18, 8	17, 22	28, 47	7, 8, 5	16, 13, 15
The corresponding drop diameters were, in millimetres				
2.1, 1.7, 2.2	2.0, 2.2	3.0, 1.9	2.0, 2.0, 2.1	1.9, 2.0, 2.6

It will be seen that in those cultures which contained more than one cell I have in many instances divided the results into two groups. In one group (A) the results are of such an order as to indicate that all the cells originally present have participated in the growth produced. In the other group (B) this is obviously not the case, and I have in these cases entered at the head of the column the number of cells which can, I think, be assumed to be responsible for the growth. The figures in brackets indicate averages of the figures below which they are placed in the table.

In Exp. 1 it will be seen that there is no evidence of any growth which might be considered allelocatalytic. The maximum growth produced by a single cell was 19, that of two cells 32, that is, less than double that of one cell.

In Exps. 2 and 3 although the medium was the same in both experiments the growth produced was very different. Possibly the small size of the drops in Exp. 2 was responsible for the slow growth. The cells in Exp. 3 were washed with Medium 2 previous to being suspended in Medium 1 in a tube closed with a cotton-wool plug in which the plug was used as a filter which did not allow many cells to pass. Possibly some growth factor was absorbed from the wool. The experiments have been recorded in view of the fact that in spite of the extremely slow growth in the one case and the extremely rapid growth in the other, there was again little evidence of allelocatalysis. The figures 70, 89, 95, 125 are subject to error in that the colonies tend to grow in three dimensions, and any number of cells greater than 50 is somewhat difficult to count with accuracy.

In Exp. 4 the maximum growth of one cell was 18, two cells 22, three cells 47. The stock culture used in this experiment had been grown in Medium 3.

In four other similar experiments, comprising 41 bacteria-free cultures, there was only one culture in which two cells produced more than double the number produced by one cell in the same time. Bearing in mind that in those cases where evidence of allelocatalysis has been observed it is possible that other cells have been present originally but have been missed in the initial count, the following conclusion seems justified:

In the case of the yeast studied the growth of two cells is, within the limits of experimental error, approximately double that of one cell provided, of course, that the conditions of growth are the same. The growth of a given yeast cell is not therefore accelerated by the presence of other living yeast cells.

If the assumption as to the number of cells which were growing in the various cultures under group B has been correct, we should also expect to

find that the growth of a single cell is not accelerated by the contiguity of other dead yeast cells. In Table II I have recorded the results obtained in a few bacteria-free cultures in which I have been fortunate to observe the growth of one cell in the presence of other cells which did not themselves grow.

Table II.

Exp. 5. Medium 3

Number of cells present after 24 hours at 25° in cultures originally containing		
1 cell	1 cell in the presence of 2 others which did not grow	
7, 18, 8	8, 7	
The corresponding drop diameters were, in millimetres		
2.1, 1.7, 2.2	2.0, 2.1	

Exp. 6. Medium 2

Number of cells present after 48 hours at 25° in cultures originally containing		
1 cell	1 cell in the presence of 4 others which did not grow	
7, 16	5, 23, 7	
The corresponding drop diameters were, in millimetres		
0.5, 0.5	0.5, 0.6, 0.6	

In Exps. 5 and 6 it will be seen that there is only one case in which the growth of one cell is accelerated by the presence of other dead cells. In two cultures in another experiment I have studied the growth of two cells in the presence of other dead cells, and there was no evidence of acceleration. The following conclusion is therefore justified.

CONCLUSION.

The growth of bacteria-free cultures of yeast in hanging-drop preparations containing one, two (or three) cells is proportional to the number of cells present, provided that all the cells are living. The presence of dead cells does not accelerate the growth. Allelocatalysis does not occur in the case of the yeast studied.

In conclusion I wish to thank Professor Peters for his kind interest and advice throughout the research, and Mr Carleton for much valuable advice in elaborating the technique adopted for the bacteriological tests.

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