52. *Physicochemical Aspects of Bacterial Growth. Part X. Observations on the Variability of Growth Rate of* Bact. Lactis Aerogenes.

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On storage for some months in a meat extract medium, a strain of *Bact. lactis aerogenes* showed in synthetic media (*a*) an increased growth rate, (*b*) an increased stationary population, and (*c*) a greater response to aeration.

The changed growth rate could not be traced to any variations in experimental technique.

Eleven sub-strains derived from single cells showed no significant differences. This shows that the strain was not sufficiently inhomogeneous to make selection a likely explanation of the change. The bacteria had apparently undergone true variation, involving a delicate readjustment of balanced intracellular reactions.

Attempts to induce variation in artificial media were negative, the growth rate in a standard medium being uninfluenced by 30—50 subcultures in various other media either favourable or unfavourable for growth.

The growth of a particular strain of *Bact. lactis aerogenes* in a synthetic medium containing, apart from the unavoidable minimal traces of impurities, only glucose, potassium dihydrogen phosphate, ammonium sulphate, sodium hydroxide, and magnesium sulphate was under observation over a period of four years. During this time, as indicated in Part VIII, certain slight, but definite differences in behaviour appeared. The time taken for the number of organisms in the above medium (aerated) to double—the "mean generation time," *m.g.t.*—decreased by about 25% : the stationary population in the aerated medium increased to about double.

That variations in growth rate do in fact occur with this organism was shown by obtaining three different strains of *Bact. lactis aerogenes* from the National Collection of Type Cultures. Of these, one (NCTC. 99) grew on the standard aerated glucose–ammonium sulphate medium with *m.g.t.* = 41 mins., another (NCTC. 240) with *m.g.t.* = 35 mins., and the third (NCTC. Morris) was only cultured with difficulty.

Changes in growth rate may arise by the operation of any or all of three causes, which will now be discussed in turn.

(1) *Selection.*—A change from slower to faster growth rates, though not the reverse, can be expected if a culture is liable at any time to produce cells which divide at an abnormal rate, since the faster-growing types will multiply more quickly and displace the slower. From the usual straight-line nature of the curves obtained by plotting the logarithms of the numbers of bacteria per c.c. (log n) against time, we may deduce that if a given bacterium has a *m.g.t.* below the average, it will tend to produce daughter cells having *m.g.t.*'s above the average, and *vice versa*. Barber (1908) and others have shown that the individual division times of bacteria are not identical. Generally, however, the variability in this respect must average out. On the other hand, as shown in Part VIII, Fig. 3, curves of log n plotted against time are not always linear, so that one cannot rely on this argument against selection by variation.

To investigate how easily selection might occur with *Bact. lactis aerogenes*, a culture grown in a medium containing a small amount of potassium cyanide which might enhance any possible inhomogeneity of the population, was plated out on nutrient agar. Discrete colonies from the agar grew after about 24 hours, and some of these, each presumed to have originated from a single bacterium, were transplanted into "heart broth," a meat extract of unknown composition. The mean generation times in the standard aerated medium of the

cultures so obtained were determined in duplicate. The results are given in Table I, where it will be seen that the average variation in the *m.g.t.*'s of all the cultures does not differ significantly from the average variation between pairs of cultures derived from the same colony. In fact, in the experiment described, no selection could be detected.

TABLE I.

Colony number.	*M.g.t.*'s in aerated $(NH_4)_2SO_4$ (mins.). (1).	(2).	Diff. of (1) and (2) halved.	Diff. from the average, 31·9. (1).	(2).
1	31·2	32·0	0·4	0·7	0·1
2	31·2	33·0	0·9	0·7	1·1
3	36·0	32·6	1·7	4·1	0·7
4	32·8	31·0	0·9	0·9	0·9
5	31·6	—	—	0·3	—
15	30·6	—	—	1·3	—
16	30·6	35·4	2·4	1·3	3·5
17	29·8	27·4	1·2	2·1	4·5
18	36·6	31·8	2·4	4·7	0·1
19	28·0	31·6	1·8	3·9	0·3
21	31·0	31·7	0·4	0·9	0·2
25	34·1	32·0	1·0	2·2	0·1

Average *m.g.t.* for all determinations = 31·9.
Mean variation of all cultures = 1·57.
Mean variation of pairs of cultures = 1·31.

(2) *Variation.*—It is well known that bacteria are able to change their characteristics of growth when stored on certain types of media. The bacteria in question had been stored on heart broth, subcultures being performed monthly. The regular transfer of the bacteria through a medium so favourable for growth might have been responsible for the change in growth rate, on account perhaps of an alteration in the relative predominance of some sets of intracellular reactions. It was thought of interest to attempt to influence this artificially. A systematic investigation of the growth rates, in a standard medium, of bacteria subcultured regularly in various favourable or unfavourable media was therefore undertaken.

Bact. lactis aerogenes was subcultured daily in several different media, and from time to time the *m.g.t.* was determined by inoculation from one of the serial cultures into the standard medium. The results, summarised in Table II, show that there is no obvious systematic change in the mean generation time with the serial number of the culture (col. 4), and that any trends lie within the average variation of growth rate determinations.

TABLE II.

Summary of experiments on serial cultures.

1. Medium in which serial culture was performed.	2. Serial culture number.	3. *M.g.t.*'s determined in the standard aerated medium (mins.).	4. Average *m.g.t.* in standard aerated medium. As shown.	Of all expts. in set.
A. Lactose, KH_2PO_4, $(NH_4)_2SO_4$, $MgSO_4$. (Unaerated.) 3 April—2 May	25	24·0, 23·6, 25·3	24·3	
	30	23·2, 28·6, 29·0	26·9	25·6
B. Glucose, KH_2PO_4, $(NH_4)_2SO_4$, $MgSO_4$. (Aerated.) (Standard medium.) 9 May—25 June	2	26·3, 26·3, 26·3	28·3	
	5	30·2, 30·4, 30·4		
	6	30·5	28·3	
	7	30·5		
	11	24·5		
	12	27·6		
	16	27·8	29·0	
	18	28·3		
	19	26·0		
	42	34·0		28·5
C. Glucose, KH_2PO_4, $(NH_4)_2SO_4$, $MgSO_4$, KCN. (Unaerated.) 15 July—20 August	6	35·0, 35·0	35·0	
	31	33·8, 33·8	33·8	
	32	33·0, 33·6	33·3	34·0
D. Heart broth, plus ethyl alcohol (2%). (Unaerated.) 1 July—1 September	15	33·3, 31·4	32·3	
	48	32·6, 35·4	34·0	33·2

On the other hand, the *average* mean generation times for the various inoculants (col. 5) show variations between themselves of significant magnitude. (It appears that, here, there was a slow change in the parent strain, kept in bouillon, from which the series A, B, C and D were derived at intervals over the period of about five months.)

(3) *Experimental Conditions.*—A carefully standardised technique was employed for the determination of bacterial growth rates. If the change apparently undergone by the bacteria is to be attributed to an alteration in experimental conditions, some quite subtle refinement must be involved. The only uncontrolled factors recognised in the experimental conditions were (*a*) the age of the inoculum, (*b*) the rate of aeration, and (*c*) the source of the air.

The effects of these factors on the growth rate were accordingly investigated. Table III shows that there is no significant change in growth rate with the age of the inoculum. Table IV shows that the rate of aeration

TABLE III.

Effect of the age of the inoculant on the mean generation time in aerated medium.

Inoculant: Unaerated heart broth.

Age of inoculant, days.	hours.	*M.g.t.* (mins.).	Average *m.g.t.*	Age of inoculant, days.	hours.	*M.g.t.* (mins.).	Average *m.g.t.*
0	5·5	31·6	30·9	9	13	29·4	31·1
0	9·5	30·2		28	12	31·8, 32·2	
				58	0	29·2, 33·8	31·5

Average *m.g.t.* = 31·2 mins.

TABLE IV.

Effect of aeration rate and air source on the mean generation time.

Mean generation times in mins.

	Fast aeration: >10 bubbles per sec.	Slow aeration: 1 bubble per sec.
Filtered fresh air from outside the laboratory	28·5	29·0
Filtered air from the compressed air supply	31·4	31·5

has no effect on the growth rate between the limits studied. All growth curves discussed above were obtained with aeration rates between these wide limits. There is, however, a small difference in growth rate evident between the two sources of air supply, which may, perhaps, be attributed to a small discrepancy in their carbon dioxide contents (Gladstone, Fildes, and Richardson, *Brit. J. exp. Path.*, 1935, **16**, 335; cf. Part II, J., 1938, 1936).

Another point which militates against the assumption of any drift in experimental conditions is that the lags, as determined in the standard medium, of inocula grown in the standard medium (*B*, Table II), all fall on the same curve when plotted against inoculant age, irrespective of the serial culture number of the inoculant (Table V).

TABLE V.

Culture ageing in, and lags determined in, the standard aerated ammonium sulphate medium.

Age of inoculum from $n = 1$, in hours.	Serial culture number.	Lag, mins.	Age of inoculum from $n = 1$, in hours.	Serial culture number.	Lag, mins.	Age of inoculum from $n = 1$, in hours.	Serial culture number.	Lag, mins.
0·5	19	580	6·5	16	117	30	42	1050
2·2	12	204	13·5	11	88	32·5	33	1195
3·5	7	242	16	5	266	36	54	1160
4·7	2	161	21	11	214	55	6	667
5·1	18	133	30	22	1380			

Finally, the greater growth rates recorded during the period April—June 1942 (Table II) cannot be due to traces of unknown constituents carried over from the heart broth, since these must have been completely eliminated during the considerable number of serial subculture operations in synthetic media.

It therefore seems that the bacteria underwent a true variation when stored in the broth. Such changes in bacteria are thought to be due to an alteration in the rather delicate balance between enzyme reactions proceeding in the cells. It appears that such changes have occurred twice during the time that this particular strain of *Bact. lactis aerogenes* has been under observation. Such changes might be expected to be capable of artificial control; several attempts have been made to exercise such control but, so far, the results have been negative.

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