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By

Chôseki FURUSAKA

Department of Agricultural Chemistry, Faculty of Agriculture, Tohoku University, Sendai, Japan.

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Cell suspensions of butyric acid forming bacteria lost their dehydrogenase activities against various substrates when washed with diluted acid such as hydrochloric or acetic acid. Even when a normal saline was used as a washing agent, they had lost their activities rapidly during the process and the storage[2].

The effects of several washing and suspending agents on the dehydrogenase activities were studied precisely. Davies and Stephenson[1] found the glucose was one of the preservation agents without decay of activities of cell suspensions for several hours.

On this paper experiments were carried out with suspensions of fairly longer time stored.

Phosphate buffers obstructed the decay torelably, but glucose was not effective on it and promoted the decay of dehydrogenation against glucose.

EXPERIMENTAL TECHNIQUES AND RESULTS.

1) *Water as a Washing and Suspending Fluid.*

Experimental Techniques: Butyric acid forming bacteria (strain B_2) were cultivated, as reported in the first paper[2], for 28 hours and then centrifuged. The harvested cell crops were washed once with distilled water and resuspended in it. The cell suspension was stored in ice chest under anaerobic condition, and at intervals samples were withdrawn, of which activities were tested against several substrates.

Results: As to be seen from Table 1, the results were summerized in the following three points.

a) Dehydrogenase activity against glucose, which was observed especially higher than others, fell into decay rapidly.

Table 1. Decay of dehydrogenase activities during the storage

		I	II	III
Phosphate Buffer		2.0 ml.	2.0 ml.	2.0 ml.
Cell Suspension		0.5	0.5	0.5
Methylene Blue M/500		0.5	0.5	0.5
Substrate		Glucose 0.5	Peptone 0.5	Water 0.5
Time of Storage	Time after the First Centrifugation	V*		
0h	2h	142	3.4	17.6
1h45′	3h45′	96	—	13.0
18h	20h	15	3.2	6.7
14h	16h	5.6**	1.3**	2.6**

* $V=1/t-1/t_{self}$, t and t_{self} =Time used for 90% decoloration with and without substrate respectively.

** Cell suspension had been left alone in room temperature under aerobic condition.

b) But dehydrogenation activity aganst peptone or self substrate, which was observed moderate, did not show remarkable decay.

c) The cells, which had been encountered into self fermentation under aerobic condition, lost especially rapidly their dehydrogenase activities against all the substrates tested.

2) *Glucose, Peptone or Phosphate Buffer as a Suspending Fluid.*

Experimental Techniques: Cells harvested from the mash, which was cultivated for 25 hours, were suspended in 25 ml. of distilled water. Then the suspension was divided into five parts and each was treated as follows.

On part 1, it was washed once with water and resuspended in water. On part 2, 5 ml. of water was added to it and it was stored in ice chest for a definite time and after this, it was washed once with water and resuspended in water. On part 3, 4 and 5, instead of 5 ml. of water, 5% glucose, 5% peptone, or M/10 phosphate buffer in equal volume were used respectively as a suspending fluid during the storage.

After the preparation of cell suspension, dehydrogenase activities were observed immediately.

Results: As to be seen from the Table 2 and 3, cells suspended and stored in glucose, peptone and distilled water lost their dehydrogenase activities very rapidly. But phosphate buffer showed remarkable resistance against əɥɟ decay of dehydrogenase activities.

Table 2. Effect of suspending fluid upon the decay of activities.

Suspending Fluid	Time of Storage	Dehydrogenase Activities (Q_{Mb}* × 10^8) against		
		Glucose M/100	Peptone 2%	Self Substrate
Dist. Water	0h	36	6.6	8.4
Dist. Water	12	6.5	1.1	0
Phosph. Buffer pH 7.0	12	46.7	2.2	6.8
Glucose 2.5%	24.5	0	2.1	0.8
Peptone 2.5%	24.5	7.3	1.3	0

Table 3. Effect of suspending fluid upon the decay of activities.

Suspending Fluid	Time of Storage h	Dehydrogenase Activities (Q_{Mb}* × 10^8) against			Nitorogen Content of the Cell %
		Glucose M/100	Peptone 2%	Self Substrate	
Dist. Water	0	109	6.2	9.2	10.6
Dist. Water	19.5	2.5	0.8	1.0	8.9
Phosph. Buffer pH 7.0	19.0	15.7	5.5	5.5	9.1
Glucose 2.5%	15.5	0.2	0.8	1.8	8.6
Pepton 2.5%	17.5	5.6	0.8	1.6	8.9

* Q_{Mb} is equivalent to the quantity of methylene blue (m-Mol) reduced by 1 mg dried cells in 1 second.

Above all it might be mentioned that the cells stored in the glucose solution lost the dehydrogenase activity against glucose exhaustively, while the cells in peptone solution retained its activity and that following the decay of dehydrogenase activity against self substrate, dehydrogenase activities against other substrates diminished very rapidly.

3) *Effects of Concentrations of Phosphate Buffer.*

As the phosphate buffer affected protectively against the decay of dehydrogenase activities, the effects of concentration of phosphate buffer on the decay were studied.

Experimental Techniques: Cells were harvested with centrifugation from 25 hours cultivated 400 ml. mash, and the cell crops were washed once with about 100 ml. of M/100 phosphate buffer and resuspended in 25 ml. of it. Then the suspension was divided into five parts. One part of them was supplied to observe the activities of the cells before strage. To each of other four parts 5 ml. of M/100, M/10, M/10 or M/20 phosphate buffer was added respectively, then stored in ice chest for a definite time. After the

storage, each part was centrifuged, washed once with M/100 phosphate buffer, resuspended in it, and then followed by activity test.

Results: The more concentrated phosphate buffer was used as a suspending fluid, the stronger resistance against the decay of the cell activities.

Table 4. Effects of concentrations of phosphate buffer on the decay.

Suspending Fluid	Time of Storage *h*	Dehydrogenase Activities ($Q_{MB} \times 10^8$) against			
		Glucose M/100	CrotonateM/20	Peptone 2%	Self Substrate
M/100 Phosph. Buff.	0	106	30.3	4.0	11.7
M/100 〃	21	21.5	6.8	2.8	3.7
M/18 〃	21	35.3	16.5	3.2	4.8
M/18 〃	24	33.2	12.8	0.8	5.0
M/36 〃	24	26.6	10.7	2.2	5.7

Cells suspended in M/100 phosphate buffer showed remarkable decay of activities.

The parallelism between the decay in dehydrogenation activities against self substrate and others were observed as in the above experiments.

DISCUSSION.

It supplies a clue to research on the cell physiology, that the cells suspended and stored in glucose lost their dehydrogenase activity against it. The correlationship between carbohydrate metabolism and nitrogen metabolism comes into consideration. The cells suspended in glucose showed the least nitrogen content after the storage, and the cells, which lost activity against self substrate, showed low activities against other substrates and also contained lower percentage of nitrogen, while the cells contained higher percentage of nitrogen, showed higher activities. Thus the cells suspended in glucose may metabolize it very vigorously at first, and this was really observed by the gas production during the storage as Davies mentioned. At this time the carbohydrate metabolism induces the nitrogen metabolism and this may excite the carbohydrate metabolism further more. After fairly long storage the nitrogenous compounds stored in the cells may be consumed exhaustively and thus the decay of activities of the cells will be resulted.

Why the phosphate buffers prevent the decay?

There are no significant evidences to interpret the reason. It may be considered that the cells just centrifuged from the cultured medium, might contained labile phosphate compounds enough to metabolize the self substrates.

And in retern this metabolism itself might supply the labile phosphates. But as all over reaction the labile phosphate and self substrate might be reduced so that the cell activities drops slowly at first and then faster. From the standpoint of chemical equilibrium the high concentration of inorganic phosphate (phosphate buffer) supplied might prevent the labile phosphate from the decomposition and lead to the minimal decay of activities of the cells.

REFERENCES

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