

CCXIII. THE LACTIC DEHYDROGENASE OF LACTIC ACID BACTERIA

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THE occurrence of lactic dehydrogenase in lactic acid bacteria has not yet been demonstrated, although many authors have already proved the presence of the dehydrogenase in animal tissues and various micro-organisms.

The specificity of the form of lactic acid dehydrogenated by the enzyme has been pointed out by several authors. Meyerhof & Lohmann [1926] observed that *d*-lactic acid was more rapidly oxidized than the *l*-acid by yeast and animal tissues including muscle, liver, kidney and brain. The same conclusion was obtained by Banga *et al.* [1932] with muscle. Green & Brosteaux [1936] observed that the enzyme of heart muscle showed complete optical specificity in oxidizing only *d*-lactic acid, while *Bact. coli* oxidized the *l*-component more rapidly and the yeast preparation preferentially attacked the *d*-component.

In the present paper, the presence of lactic dehydrogenase was verified in all the kinds of lactic acid bacteria under investigation, and the enzymes of these bacteria were found to show complete optical specificities.

Methods

The various kinds of lactic acid bacteria were cultivated on 600 ml. koji extract containing 24–30 g. sugar and 30 g. CaCO₃ at 30° for 5 days. The bacterial cells were collected on the centrifuge after being separated from calcium carbonate by decantation. The cells were then suspended in 100 ml. water and strongly aerated. After being washed several times with water, the cells were finally suspended in 50 ml. of water and again aerated.

For the detection of dehydrogenase, the reduction time of methylene blue at 40° was measured anaerobically (less than 20 mm.) in a Thunberg tube into which 0.2 ml. of the bacterial suspension (in the case of *Lactob. sake* 0.4 ml. was used) was put with 0.2 ml. 5% Ca lactate solution and 1.8 ml. methylene blue solution prepared by mixing 8 ml. 0.05% methylene blue and 6 ml. of 0.2 *M* phosphate buffer adjusted to pH 7.2.

Lactobacillus sake (d-acid-former)

When the experiments were carried out with *Lactob. sake* No. 84, it will be seen in Table I that reduction of methylene blue was observed in the presence

Table I. *Specificity of lactic dehydrogenase*

Donator	Reduction time of methylene blue (min.)		
	<i>d</i> -Acid-former	<i>l</i> -Acid-former	<i>dl</i> -Acid-former
<i>d</i> -Lactic acid	21	>180	25
<i>l</i> -Lactic acid	>180	11	13
<i>dl</i> -Lactic acid	20	17	15
Water (control)	>180	>180	>180

of *d*- and *dl*-lactic acids. Therefore the bacterial cells were found to show complete optical specificity in oxidizing only *d*-lactic acid, as was already pointed out by Green & Brosteaux [1936] with heart muscle extract.

Leuconostoc mesenteroides (*l*-acid-former)

In contrast with the case of *Lactob. sake* mentioned above, only the *l*-component of lactic acid was available to *Leuconostoc mesenteroides* var. *sake* as donator (see Table I).

It is of interest that the optical specificities of the lactic acids dehydrogenated by these bacterial cells coincide with those of the acid produced by the bacteria themselves. Lactic dehydrogenase can therefore be classified into *d*- and *l*-enzymes.

Lactobacillus plantarum (*dl*-acid-former)

It will be seen in Table I that *Lactob. plantarum* sp. dehydrogenated all the optical components of lactic acid, although the *l*-component was attacked a little more rapidly.

Thus all the kinds of lactic acid bacteria mentioned above effected dehydrogenation of lactic acid in the presence of methylene blue. No oxidation of lactic acid was ever observed in the absence of methylene blue.

In order to dehydrogenate all the optical components of lactic acid, the presence of both *d*- and *l*-lactic dehydrogenases would not be absolutely necessary, since all forms of the acid could be attacked by any one of the dehydrogenases in presence of racemiase, with which the racemic form of lactic acid was always produced as has already been pointed out by us [1937].

It was found by us [1938] that racemiase in *Lactob. plantarum* was easily inactivated by treatment with acetone, while lactic dehydrogenase would not be inactivated by acetone, as was pointed out by Harden & Macfarlane [1931] with yeast. Therefore the experiments shown in Table II were carried out with acetone-dried bacteria in order to investigate the effect of racemiase upon the dehydrogenation of lactic acid.

Table II. *Effect of racemiase*

<i>Lactob. plantarum</i> <i>dl</i> -Acid-former	Reduction time of methylene blue (min.)		
	<i>d</i> -Lactic acid	<i>l</i> -Lactic acid	Water
Resting cells	38	18	>180
Acetone-dried cells	>180	20	>180

It will be seen in Table II that acetone-dried cells never attacked *d*-lactic acid, while *l*-acid was as easily dehydrogenated by it as by the resting cells.

The reason why *Lactob. plantarum* attacked all the forms of lactic acid was thus attributable not to the presence of both *d*- and *l*-lactic dehydrogenases, but to that of *l*-lactic dehydrogenase co-operating with racemiase.

The product of oxidation

The identification of pyruvic acid produced by oxidation of lactic acid with lactic dehydrogenase is not easy to accomplish, since decomposition of the pyruvic acid by carboxylase usually occurs or α -ketonoxidase is present in the dehydrogenase preparations.

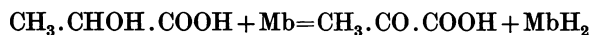
In the present experiments, it was found that pyruvic acid could be directly isolated when lactic acid bacteria were used for the dehydrogenase.

(a) *Lactobacillus plantarum*. The experiments were carried out with seven large (100 ml.) Thunberg tubes. Each tube, containing 10 ml. 0.2 *M* Na *dl*-lactate, 10 ml. 0.2 *M* phosphate buffer (*pH* 7.2), 20 ml. 0.5% methylene blue and 20 ml. bacterial suspension, was evacuated and kept at 40°. When the methylene blue was almost reduced, another 20 ml. of the methylene blue solution were added and the experiments were continued in exactly the same manner until decoloration of the dye was complete, which was attained in about 2 hr. throughout the whole of the experiments.

The decolorized solutions were collected, the dye adsorbed first with kaolin and then with active C. The clear solution thus obtained was acidified with H₂SO₄ and the pyruvic acid extracted by ether.

0.4 g. of yellowish acicular crystals, *M.P.* 184°, was obtained by the addition of phenylhydrazine hydrochloride solution to the aqueous solution of the acid after removal of the ether. After recrystallization the *M.P.* was found to be 192° which was identical with that of the phenylhydrazone of pyruvic acid. (Found: C, 60.49; H, 5.73%. C₉H₁₀O₂N₂ requires C, 60.62; H, 5.62%.)

The yield was 51% of that calculated from the equation:



(b) *Lactobacillus sake*. The experiments were carried out in the same manner as was mentioned above, with two Thunberg tubes in which were placed 10 ml. 0.2 *M* Na *d*-lactate, 10 ml. 0.2 *M* phosphate buffer, 5 ml. 0.5% methylene blue and 20 ml. of the bacterial suspension.

The solution was analysed after 5 hr., and 0.016 g. of the phenylhydrazone of pyruvic acid was obtained; yield found, 60%.

(c) *Leuconostoc mesenteroides* var. *sake*. The same experiments as were instituted with *Lactob. sake* were carried out with *l*-lactate. The decoloration of the 15 ml. 0.5% methylene blue in each Thunberg tube was attained within 6 hr. but the tubes were kept for a further 6 hr., in order to test whether pyruvic acid would be decomposed by the bacteria.

The yield of pyruvic acid was observed to be 58%, since 0.049 g. of the phenylhydrazone was obtained from the two tubes.

No evidence of the production of acetaldehyde or acetic acid, which would be derived from pyruvic acid by carboxylase or α -ketonoxidase, was ever obtained in these experiments.

Thus it was clearly demonstrated that lactic dehydrogenase existed in all the lactic acid bacteria and that carboxylase could not be detected even in *Leuconostoc*, which converts glucose into lactic acid, alcohol and CO₂. This is in disagreement with the suggestion of Peterson *et al.* [1922] that the heterofermentation revealed by such types of bacteria as *Leuconostoc* would be caused by the presence of carboxylase.

SUMMARY

1. The occurrence of lactic dehydrogenase was verified with various kinds of lactic acid bacteria.

2. It was found that a *d*-acid-former (*Lactob. sake*) dehydrogenated only *d*-lactic acid and an *l*-acid-former (*Leuconostoc*) attacked only the *l*-acid. Lactic dehydrogenase is therefore considered to be a mixture of *d*- and *l*-enzymes.

3. The reason why a *dl*-acid-former (*Lactob. plantarum*) attacked all forms of lactic acid was not the presence of both kinds of lactic dehydrogenases, but the co-operation of racemiasse with the *l*-enzyme.

4. The occurrence of carboxylase was never detected and pyruvic acid was easily isolated, in a yield of about 60%, as the oxidation product of lactic acid by any of the bacteria used.

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