

## STUDIES ON THE ANAEROBIC MICROCOCCI

### II. THE FERMENTATION OF LACTATE BY MICROCOCCUS LACTILYTICUS

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*Micrococcus lactilyticus* is a strictly anaerobic microorganism that is indigenous to the mouth of man and possibly other animals. The metabolism of this organism is interesting in that it fails to ferment sugars but carries out a vigorous fermentation of pyruvate, malate, and lactate (Foubert and Douglas, 1948). Lactate fermentation in the absence of an additional hydrogen acceptor is rare among bacteria but has been found to occur in *Propionibacterium* (van Niel, 1928) and *Butyribacterium* (Barker and Haas, 1944).

This paper presents the results of quantitative experiments to determine the nature of the lactate fermentation conducted by *M. lactilyticus*.

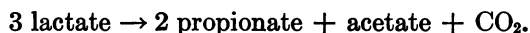
#### METHODS

The results reported here were obtained with one strain (T5) of *M. lactilyticus*. The fermentations were conducted at 37 C in an all-glass fermentation vessel of the type designed by Professor H. A. Barker of the University of California. The medium used contained 0.2 per cent Difco yeast extract and 1 per cent sodium lactate. Under these conditions the fermentations were complete in 24 hours, and the bacteria utilized 3.5 to 4.0 mM of lactate per 100 ml of medium.

The fermentation gases were collected over mercury and analyzed volumetrically. Lactate was determined by the method of Barker and Summerson (1941). Volatile acids were determined quantitatively by the double distillation method of Friedemann (1938). The identity of the volatile acids was tentatively determined by the method of Osburn, Wood, and Werkman (1936) and verified by partition chromatography. The chromatographic method used was essentially a combination of the procedures of Elsdon (1946) and Ramsey and Patterson (1945). The individual acids, after resolution on silica gel, were chromatographed with known acids to establish their identity.

#### RESULTS

The only fermentation products produced in detectable amounts from lactate by *M. lactilyticus* are propionic and acetic acids, carbon dioxide, and hydrogen. Table 1 summarizes the results of a typical fermentation. The fermentation can be seen to be fairly similar to that carried out by the propionic acid bacteria, but it differs in that hydrogen gas is produced. The ratio of propionate to acetate is considerably less than 2.0, the value required by the equation for the propionic acid fermentation of lactate:



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This is to be expected, however, for on theoretical grounds the formation of each mole of hydrogen gas should spare the reduction of 1 mole of lactate to propionate. The data in Table 1 bear this out; the ratio, propionate + hydrogen to acetate, is 1.99.

TABLE 1  
*Fermentation of lactate by Micrococcus lactilyticus (T5)*  
(mM per 100 mM lactate fermented)

Propionic acid.....		63.7
Acetic acid.....		39.5
Carbon dioxide.....		39.1
Hydrogen.....		14.2
Carbon recovery (%).....	103.6	
O/R index.....	0.99	

As cell suspensions were shown to have no action upon formate, the fermentation can be postulated to occur according to the following over-all reactions:



Hydrogen formation probably occurs by a diversion to hydrogen gas (equation 2) of part of the available hydrogen produced in the oxidative phase of the fermentation (equation 1), whereas propionate can be considered to arise from the reduction of lactate (equation 3).

#### SUMMARY

*Micrococcus lactilyticus* carries out a propionic acid fermentation of lactate, which differs from the classical propionic acid fermentation in that hydrogen gas is produced.

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