TRACER STUDIES ON THE ROLE OF ACETIC ACID AND CARBON DIOXIDE IN THE FERMENTATION OF LACTATE BY CLOSTRIDIUM LACTO-ACETOPHILUM

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In a previous study of the fermentation of lactate by *Clostridium lacto-acetophilum* (Bhat and Barker, 1947) it was shown that pure cultures of this organism can decompose lactate only when acetate is provided as a second substrate, whereas enrichment cultures of the same organism do not require added acetate. This difference in nutritional requirements indicated a corresponding difference in the catabolic processes occurring in the two types of cultures. In pure cultures, lactate and acetate disappeared while butyric acid, carbon dioxide, and hydrogen were formed, whereas in enrichment cultures lactate was decomposed with the formation of acetate in addition to the other products. It is also significant that the yield of carbon dioxide was much lower in the enrichment cultures. The reduced yield of carbon dioxide taken in conjunction with the formation of acetate indicated that the organisms in the enrichment cultures were using carbon dioxide as an oxidant and were converting it to acetic acid. In pure cultures the high yield of carbon dioxide and the requirement for acetate indicated that the bacteria were unable to reduce carbon dioxide in this way.

The tracer experiments described in the present paper were designed to provide a direct test of the conclusions derived from the above-mentioned nutritional and metabolic experiments.

EXPERIMENTAL RESULTS

In the first experiment (table 1, experiment 1) lactate was fermented by an enrichment culture in the presence of C^{14}-labeled bicarbonate. At the end of the fermentation, the isotope was found in acetate, butyrate, and the residual bicarbonate. This proves that the bacteria converted carbon dioxide to acetate.[2] The data also indicate that this was a quantitatively important reaction in the fermentation. The labeled butyrate was undoubtedly formed from labeled acetate, as is demonstrated in experiment 3 (table 1).

In the second experiment (table 1, experiment 2) lactate was fermented by a pure culture of *C. lacto-acetophilum* in the presence of labeled bicarbonate. Only a relatively insignificant amount of the labeled carbon was found in the

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[2] The distribution of C^{14} in the fatty acids was not determined, but in view of the results obtained with other bacteria (Barker, Kamen, and Haas, 1945) it is probable that all the carbon atoms were labeled.

volatile acids. This confirms the previous conclusion that pure cultures cannot use carbon dioxide as an oxidant.

The third experiment (table 1, experiment 3) consisted of a pure culture fermentation of lactate in the presence of labeled acetate. The butyrate formed in the fermentation was radioactive, showing that it had been formed from acetate. Furthermore, the specific activity of the acetate was greatly reduced during the fermentation. This proves that acetate was formed from lactate even though there was a net disappearance of acetate. From the magnitude of the change in specific activity it was calculated by the method previously described (Barker, Kamen, and Haas, 1945) that approximately one mole (actually 0.91 mole) of acetate was formed per mole of lactate decomposed. This is consistent with the view that all the lactate undergoes an oxidative decarboxylation. The slight radioactivity in the final bicarbonate shows that little or no oxidation of acetate occurred.

TABLE 1

Carbon dioxide and acetate utilization by pure and enrichment cultures of C. lacto-acetophilum

	EXPERIMENT 1 ENRICHMENT CULTURE		EXPERIMENT 2 PURE CULTURE		EXPERIMENT 3 PURE CULTURE	
	mM/100 ml	cts/min/mM	mM/100 ml	cts/min/mM	mM/100 ml	cts/min/mM
Substrates						
Lactate decomposed	8.50		3.74		6.77	
Acetate, initial			6.20		4.38	5,100
Bicarbonate, initial	1.40	10,600	0.53	10,700		
Products						
Acetate, final	3.90	1,350	2.52	65	1.76	1,450
Butyrate, final	4.29	1,650	3.28		4.59	4,250
Bicarbonate, final	2.62	570	3.97	1,220	6.47	40
Hydrogen formed	12.5		2.48		4.83	

The experimental results demonstrate that there is a striking difference in the chemical reactions occurring in enrichment and pure cultures of *C. lacto-acetophilum;* the former utilize carbon dioxide for the synthesis of acetic acid whereas the latter do not. So far we do not know the reason for this difference. The most obvious explanation is that the enrichment cultures contain one or more organisms that are able to utilize carbon dioxide. As yet, however, all attempts to isolate bacteria from the enrichment cultures which either alone or in combination with *C. lacto-acetophilum* can reduce carbon dioxide and, consequently, ferment lactate in the absence of added acetate have been unsuccessful. *Butyribacterium rettgeri* can cause a fermentation of this type (Barker, Kamen, and Haas, 1945), but it has never been found in these cultures.

EXPERIMENTAL PROCEDURES

The experiments were done with growing cultures. In the pure culture experiments, *C. lacto-acetophilum*, strain 3, was used (Bhat and Barker, 1947).

In the enrichment culture experiment, the sterilized medium was inoculated with garden soil. All cultures were incubated at 37 C until growth ceased.

The basal medium contained the following compounds in grams per 100 ml of glass-distilled water: sodium lactate, 1.0; yeast autolyzate, 0.3; sodium thioglycolate, 0.05; $(NH_4)_2SO_4$, 0.05; K_2HPO_4, 0.1; $MgSO_4 \cdot 7H_2O$, 0.01; $FeSO_4 \cdot 7H_2O$, 0.002; and $CaSO_4 \cdot 2H_2O$, 0.001; pH 7.0. To this medium was added synthetic carboxyl-labeled acetate or labeled sodium carbonate in the amounts indicated in table 1. The acetate was added before autoclaving, whereas the sodium carbonate was added afterward as a sterile solution. The fermentations were conducted in an all-glass vessel with an outlet for collecting the evolved gases over mercury. Oxygen was excluded by the use of an "oxsorbent" seal.

The fermented media were analyzed by methods previously used in this laboratory (Bornstein and Barker, 1948). Acetic and butyric acids were separated by azeotropic distillation. Radioactivity measurements were made by the technique described by Kamen (1947).

SUMMARY

By the use of C^{14} it has been shown that the bacteria in enrichment cultures of *Clostridium lacto-acetophilum* use carbon dioxide as an oxidant, converting it into acetic and butyric acids. In pure culture this organism is unable to reduce carbon dioxide, but it oxidizes lactate to acetate and carbon dioxide and then converts the acetate to butyrate.

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