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S. KUMÉ
K. OTOZAI
H. WATANABÉ

Faculty of Science, Osaka University,
Osaka. July 20.

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Use of 'Amberlite IRC-50' for the Partial Purification of a Bacterial Pectinase

In the course of an investigation of the pectolytic enzymes of certain plant pathogens, it was found possible to effect a partial purification of bacterial pectinase with a cation exchange resin, 'Amberlite IRC-50'.

A separation of pectinesterase and polygalacturonase with 'Amberlite IR-100' has been reported by McCulloch and Kertesz¹, though the validity of their method has been questioned by Lineweaver, Jang and Jansen². Their objection to the use of Amberlite IR-100, namely, the development within the column of rather low pH values (pH 1.0–2.0), was eliminated in the present investigation by using 'Amberlite IRC-50', which is a weak-acid cation exchanger readily buffered at any desired pH value over a wide range.

The pathogen, *Bacterium aroideae*, was grown in a liquid medium of the following composition: glucose 1, peptone 0.5, potassium dihydrogen phosphate 0.08, magnesium sulphate 0.03, calcium carbonate 1.0, water to 100 per cent. After five days, the culture fluid was centrifuged at 4,000 r.p.m. for 20 min. to remove most of the bacteria, excess carbonate, etc., and the pH adjusted to 7.6.

A suspension of the sodium-salt form of the resin was treated with N/10 hydrochloric acid to bring the pH to 7.6–7.8, and then allowed to settle as a sediment to form a column 20 cm. × 1 cm. The system was such that no further buffering was required.

In a typical test, 50 ml. of centrifuged culture filtrate was passed through the column at an effluent rate of about 1.25 ml./min. After thorough washing of the column with distilled water, the adsorbed enzyme was eluted with 15 ml. of N/10 hydrochloric acid, the effluent rate being 1.0 ml./min., and the pH of the effluent about 6.2–6.4. It should be pointed out that though N/10 acid is used as an elutant, it is rapidly neutralized by exchange with sodium ions from the resin, and in view of the slow rate of flow, the pH of the major part of the column probably does not fall below 6.0. Sodium chloride was removed from the enzyme solution by dialysis through 'Cellophane'. 1 ml. of the dialysed solution caused complete hydrolysis of 5 ml. of 1 per cent sodium pectinate solution in 15 min. This was of the same order as a dialysed preparation of the crude culture fluid, so that although considerable quantities of impurities are removed in the process there is an appreciable loss of pectolytic enzyme.

Further details of the investigation are to be published elsewhere.

P. W. TALBOYS

Department of Botany, Royal College of Science,
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Reduction by Molecular Hydrogen of Acetoacetate to Butyrate by Butyric Acid Bacteria

Stadtman and Barker^{1,2}, working with extracts of *Cl. kluyveri*, and Cohen and Cohen-Bazire^{3–5}, using suspensions of butyric acid bacteria, found that acetoacetate is split into two C₂-carbon residues. These workers found acetate from acetoacetate, whereas Stadtman and Barker observed a phosphoroclastic splitting of acetoacetate to acetate and acetyl phosphate. It should be noted that the suspensions utilized by Cohen and Cohen-Bazire possess strong acetyl phosphatase activity. Nisman⁶, utilizing extracts of the same bacteria obtained by ultrasonic vibration, finds that pyruvate yields considerable amounts of acetyl phosphate.

The reverse reaction, that is, the synthesis of acetoacetate from acetate and acetyl phosphate, has been postulated by Lipmann⁷ as a step in the synthesis of butyrate from pyruvate. Stadtman and Barker², in an attempt to achieve this reaction, find only small but none the less significant amounts of a β-keto acid which they think to be acetoacetic acid. β-Keto acid accumulates in greater quantity in presence of 0.002 M hydrogen cyanide. Incubation of extracts of *Cl. kluyveri* with acetate, acetyl phosphate and hydrogen leads to a synthesis of butyrate⁸, whereas acetoacetate incubated with hydrogen under the same conditions gives rise, almost mole per mole, to β-hydroxybutyrate, which is not further metabolized, and to traces of butyrate. In view of these results, Stadtman and Barker² come to the conclusion that acetoacetate is not a precursor of butyrate. This assumption is supported by further experiments conducted by the same workers with carbon-14 labelled compounds.

On the other hand, we^{4,5} have shown that suspensions of butyric acid bacteria may utilize lactate or pyruvate as hydrogen donors for the transformation of acetoacetate to butyrate. With 'deficient' suspensions⁹, pyruvate or acetoacetate alone give rise only to acetate; simultaneous incubation with both pyruvate and acetoacetate leads to the formation of butyrate. In view of Stadtman and Barker's experiments, it was interesting to study the reduction of acetoacetate by molecular hydrogen. Since we have found β-hydroxybutyrate to be fermented *in vacuo* to acetate and butyrate^{4,10}, we have also studied its behaviour in a hydrogen atmosphere. Our results are summarized in Table 1.

It is seen from these results that, contrary to what occurs *in vacuo*^{4,10}, β-hydroxybutyrate is not metabolized in an atmosphere of hydrogen. This is consistent with Stadtman and Barker's observations. In

Table 1. Suspensions are prepared as previously described (ref. 9). Each Warburg vessel contained in the main part phosphate buffer M/45, substrate and water to adequate volume; 0.2 ml. 20 per cent potassium hydroxide in the central well; 0.5 ml. bacterial suspension in the side bulb. Total volume: 3.2 ml. Suspension and substrate are mixed after 15 min. equilibration. Temperature: 37° C.; pH 7; gas phase, hydrogen. The data have been corrected for hydrogen absorption of the suspension alone. The results are expressed in micromoles

Strain	Acetoacetate		Δ Acetoacetate	Hydrogen	β-Hydroxybutyrate	
	Initial	Final			Initial	Final
<i>Cl. acetobutylicum</i> PC48	43	0	43	32	—	11.3
"	—	—	—	0	13.7	13.5
<i>Cl. saccharobutylicum</i> GR4	—	—	—	0	13.7	13.4