For these reasons we consider the arabinosidase to be a separate entity, not identical with either the xylanase or the xylobiase. This enzyme, like the other two, is inducible but in a curious way. It is not produced during growth on monosaccharides, but appears whenever either of the other two enzymes is formed. Hence it is produced during growth on xylobiose or rhodymenan, neither of which contains arabinose. Nothing further can be said at present about the properties of this enzyme, except that its electrophoretic mobility is intermediate between those of xylanase and xylobiase.

SUMMARY

1. Two strains of xylan-fermenting bacteria, one from the sheep and the other from the bovine rumen, have been grown in media containing a wide variety of xylans and related carbohydrates. Cell-free extracts have been prepared and tested for hydrolytic activity.

2. Two enzymes have been separated from the whole extracts by zone electrophoresis in starch columns.

3. The less mobile enzyme, referred to as xylanase, is formed by the two bacteria when these are grown on xylan or similar polysaccharide. It acts upon xylan by hydrolysing inner linkages, producing xylobiose as the main end product. It hydrolyses oligosaccharides similarly but has negligible action on xylotriose and xylobiose. Its effect on the xylan is inhibited by the presence of arabinose side chains.

4. The electrophoretically more mobile enzyme, referred to as xylobiase, is produced by the two bacteria when these are grown on xylose polysaccharides or xylobiose. It is a non-specific β -xylosidase, acting upon oligosaccharides by removal of single xylose units, and also hydrolyses phenyl β -xyloside. When acting upon a 20% (w/v)

solution of xylobiose, this enzyme synthesizes small amounts of two trisaccharides, xylotriose and 3²- β -xylosylxylobiose.

5. Some evidence is presented for the existence in the crude enzyme preparations of a discrete arabinosidase, which removes the arabinose side chains present in soluble pentosans. It appears to be formed by the bacteria whenever either xylanase or xylobiase is produced.

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REFERENCES

- Allen, P. J. & Bacon, J. S. D. (1956). *Biochem. J.* **63**, 200.
 Bealing, F. & Bacon, J. S. D. (1953). *Biochem. J.* **53**, 277.
 Bishop, C. T. & Whitaker, D. R. (1955). *Chem. & Ind.* p. 119.
 Bryant, M. P. & Small, N. (1956). *J. Bact.* **72**, 16.
 Conchie, J. (1954). *Biochem. J.* **58**, 552.
 Crook, E. M. & Stone, B. A. (1957). *Biochem. J.* **65**, 1.
 Doetsch, R. N., Howard, B. H., Mann, S. O. & Oxford, A. E. (1957). *J. gen. Microbiol.* **18**, 156.
 Drury, H. F. (1948). *Arch. Biochem.* **19**, 455.
 Festenstein, G. N. (1958a). *Biochem. J.* **69**, 562.
 Festenstein, G. N. (1958b). *Biochem. J.* **70**, 49.
 Howard, B. H. (1957). *Biochem. J.* **67**, 643.
 Hungate, R. E. (1950). *Bact. Rev.* **14**, 1.
 Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). *J. biol. Chem.* **193**, 265.
 McIlvaine, T. C. (1921). *J. biol. Chem.* **49**, 183.
 Mould, D. L. & Stephen, J. (1958). *Biochem. J.* **69**, 44F.
 Mould, D. L. & Thomas, G. J. (1958). *Biochem. J.* **69**, 327.
 Nelson, N. (1944). *J. biol. Chem.* **153**, 375.
 Perlin, A. S. (1951). *Cereal Chem.* **28**, 382.
 Porter, W. L. & Hoban, N. (1954). *Analyt. Chem.* **26**, 1846.
 Sørensen, H. (1957). *Acta agric. scand.* Suppl. 1.

APPENDIX

Isolation and Properties of the Xylan-Fermenting Bacterium 11

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The isolation and properties of the bacterium 11 (Institute collection) used in the preceding study of the degradation of xylans are described here.

METHODS

The medium used for isolation was the same as that described in the above paper for maintenance of stock

cultures, but without yeast extract and containing wheat-flour pentosan (0.25%). It was later found that better growth occurred with yeast added. Since growth occurred on this medium without added carbohydrate the medium used for determining the substrates utilized was altered in that the rumen fluid was replaced by Bacto-tryptone (0.2%) and Bacto-peptone (0.2%). In the presence of a suitable substrate this medium gave growth about equal to that of the rumen fluid medium. Very little growth

occurred on this medium in the absence of utilizable carbohydrate. Substrates were added as sterile solutions (sterilized by filtration, except for polysaccharides which were sterilized by steaming) to give a final concentration of 0.5 or 1%. Utilization of substrate was tested by comparing the growth on slopes of the appropriate medium with growth on the basal medium, after one subculture on the substrate-containing medium. A maximum incubation time of 8 days was used to see if growth occurred. Colonies were tested with iodine solution after 24 and 48 hr. to determine whether formation of intracellular starch-like polysaccharide occurred during growth of the cells. Tests for motility and other properties were made by the methods described by Doetsch, Howard, Mann & Oxford (1957). For determination of the products of fermentation of xylose the rumen fluid in the liquid medium described in the paper above was replaced by water, leaving the yeast extract (0.5%). The cells were centrifuged off before analysis of the supernatant fluid. Total volatile fatty acids were determined by titration of a steam-distillate, after acidification. Qualitative tests for the presence of different volatile fatty acids were made by paper chromatography (Elsden & Lewis, 1953; Burness & King, 1958). Quantitative determinations were made by chromatography on silicic acid columns (Keeney, 1955). Formic acid was determined colorimetrically (Grant, 1947), and by difference after distilling the initial volatile acid distillate with mercuric salts (Friedemann, 1938). Lactic acid was determined on the original culture supernatant by the method of Barker & Summerson (1941). Succinic acid was not determined.

Isolation of the organism. Rumen fluid, taken via a permanent rumen cannula from a heifer 4 hr. after feeding on grass silage, was diluted tenfold by the use of the anaerobic technique and diluting fluid described by Doetsch, Robinson & Shaw (1952); glucose was omitted from the diluting fluid. A portion (0.5 ml.) of diluted sample was transferred to 5 ml. of liquid rumen fluid-pentosan-agar medium in test tubes and the medium allowed to solidify with the stoppered tubes laid flat. Colonies from the primary medium were subcultured on to slopes of the same medium. The growth on these slopes was usually mixed and so the dilution procedure was repeated until a pure culture was obtained.

RESULTS

Streptococcus bovis grew along with curved Gram-negative rod-like bacteria in up to 10^6 – 10^8 dilutions of the rumen fluid. From these curved rods three isolates were selected for further study, one being that numbered 11. The properties of this bacterium differed somewhat from those of the other two, but only this one will be described here.

Properties of the organism. The bacterium was non-motile under the conditions of test and flagella were not observed. The bacterium was a strict anaerobe, no growth taking place on media in which the resazurin was oxidized. Cells were Gram-negative, usually curved and $0.3 \mu \times 1.8 \mu$, occurring singly or sometimes in short chains. The cells were somewhat pleomorphic and in older cultures filamentous forms (up to 6.5μ) and bulbous forms appeared. No intracellular starch-like polysaccharide was detected. Colonies on pentosan-agar medium were whitish, round, smooth and opaque. Hydrogen sulphide was formed, but catalase and indole tests were negative. Growth occurred at 29° and 38° , but not at 55° .

Substrates on which growth occurred are shown in Table 1. Since the media contained carbonate no evaluation of metabolic gas production was made. The proportions of the products of fermentation of xylose varied somewhat with rate of growth etc. in different experiments, but formic acid, acetic acid, propionic acid and butyric acid were formed, in one experiment in the proportions 35, 13.9, 26.9 and 17.2% of the total volatile acids formed. Lactic acid was also produced, in one experiment in a concentration of 0.144 m-equiv./5 ml. of culture, compared with a total volatile-acid concentration of 0.160 m-equiv./5 ml. of culture. These concentrations accounted for 69.5% of the carbon of the xylose originally present in the medium.

Table 1. *Fermentation reactions of rumen organism 11*

The medium is described in the text. All substrates were at 1% concentration except those marked with an asterisk, which were at 0.5%. +, – and $\pm$: Growth, no growth and little growth respectively, in the presence of substrate.

Substrate	Reaction	Substrate	Reaction	Substrate	Reaction	Substrate	Reaction
D-Xylose	+	Glycerol	–	Cellobiose	+	Amylose*	–
L-Arabinose	+	Ribitol*	–	Trehalose	+	Starch	–
D-Ribose	–	D-Mannitol	$\pm$	Melibiose	+	Cellulose	–
L-Rhamnose	+	D-Sorbitol	+	Raffinose	+	D-Glucurono-lactone*	–
L-Fucose*	+	Dulcitol	+	Methyl α -D-glucoside	+	Pyruvate	+
D-Glucose	+	meso-Inositol	–	Salicin	+	DL-Lactate	–
D-Mannose	+	D-Glucosamine-HCl	–	Aesculin	+	Fumarate	–
D-Galactose	+	Sucrose	+	Inulin	–	Succinate*	–
D-Fructose	+	Maltose	+	Dextrin	+	Citrate	–
L-Sorbose	–	Lactose	+	Glycogen	–	D-Glucose 1-phosphate	–

DISCUSSION

Bacterium 11 differs from *Bacteroides amylogenes* (Doetsch *et al.* 1957) in some of its fermentation reactions, and the fact that it does not form an iodophilic polysaccharide excludes it from classification with this organism. Bryant and his co-workers have described a number of Gram-negative rod-like bacteria isolated from bovine-rumen contents, and bacterium 11 could be classified as a strain of *Butyrivibrio fibrisolvens* (Bryant & Small, 1956*a*). The fact that it rapidly ferments xylan precludes it from inclusion with the species *Succinivibrio dextrinsolvens* (Bryant & Small, 1956*b*) and *Bacteroides succinogenes* (Hungate, 1950), and suggests its inclusion with *Butyrivibrio fibrisolvens*. Although the type species of *B. fibrisolvens* was motile, it was suggested that the 'less actively cellulolytic rod' of Hungate was a *Butyrivibrio* species and this was non-motile (Bryant & Small, 1956*a*). The fermentation reactions of *B. fibrisolvens* strains are variable, but are generally similar to those of bacterium 11, and all are non-iodophilic.

Bacterium 11 differs from *Bacteroides amylogenes* in the products of fermentation of xylose, especially in the formation of lactic acid. However, a lactic acid fermentation of xylose is known, for instance with *Lactobacillus pentosus* (Gest & Lampen, 1952). Although lactic acid is not a final product of the fermentation of xylose by mixed rumen organisms

(Doetsch, Robinson, Brown & Shaw, 1953), the work of Pazur, Shuey & Georgi (1958) suggests that it might be an intermediate product.

SUMMARY

The isolation and properties of a xylanfermenting bacterium from the rumen are described. It was tentatively assigned to the genus *Butyrivibrio*.

REFERENCES

- Barker, S. B. & Summerson, W. H. (1941). *J. biol. Chem.* **138**, 535.
 Bryant, M. P. & Small, N. (1956*a*). *J. Bact.* **72**, 16.
 Bryant, M. P. & Small, N. (1956*b*). *J. Bact.* **72**, 22.
 Burness, A. T. H. & King, H. K. (1958). *Biochem. J.* **68**, 32*P*.
 Doetsch, R. N., Howard, B. H., Mann, S. O. & Oxford, A. E. (1957). *J. gen. Microbiol.* **16**, 156.
 Doetsch, R. N., Robinson, R. Q., Brown, R. E. & Shaw, J. C. (1953). *J. Dairy Sci.* **36**, 825.
 Doetsch, R. N., Robinson, R. Q. & Shaw, J. C. (1952). *J. Anim. Sci.* **11**, 52.
 Elsdon, S. E. & Lewis, D. (1953). *Biochem. J.* **55**, 183.
 Friedemann, T. E. (1938). *J. biol. Chem.* **123**, 161.
 Gest, H. & Lampen, J. O. (1952). *J. biol. Chem.* **194**, 555.
 Grant, W. M. (1947). *Analyt. Chem.* **19**, 206.
 Hungate, R. E. (1950). *Bact. Rev.* **14**, 1.
 Keeney, M. (1955). *Univ. Maryland Misc. Publ.* **238**, 23.
 Pazur, J. H., Shuey, E. W. & Georgi, C. E. (1958). *Arch. Biochem. Biophys.* **77**, 387.

The Structure of a Disaccharide Liberated by Lysozyme from the Cell Walls of *Micrococcus lysodeikticus*

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Salton (1956) studied in some detail the components produced by the action of egg-white lysozyme on the isolated cell walls of certain Gram-positive micro-organisms. In addition to several large fragments which would not diffuse through cellophan, he found that a considerable proportion of the digest was diffusible. He stated that the main 'small fragment' present in the diffusible fraction was possibly a disaccharide of *N*-acetylglucosamine and the unknown amino sugar present in many cell walls (subsequently characterized, e.g. Strange & Kent, 1959, as muramic acid), and adduced certain evidence to support this idea. The work described here confirms the presence of such an oligosac-

charide in lysozyme digests of cell wall. The supposition that it is a disaccharide is found to be correct and its structure is established.

EXPERIMENTAL

Methods

Isolation of walls and preparation of lysozyme digests. *Micrococcus lysodeikticus* (N.C.T.C. 2665) was grown in surface culture and the cell walls were isolated as previously described (Perkins & Rogers, 1959). For preparation of digests the isolated walls (100 mg.) were suspended in 0.1*M*-ammonium acetate buffer, pH 6.1 (25 ml.), and incubated at 37° with lysozyme (crystalline; The Armour