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NOTES

Small Pectinolytic Spirochetes from the Rumen

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Two strains of small spirochetes utilizing pectin as sole source of energy for growth were isolated from the rumen of cattle and partially characterized. The pectinolytic enzyme produced by one of the organisms was classified as a poly-1,4-α-D-galacturonide lyase (EC 4.2.2.2). A pectinesterase (EC 3.1.1.11) was also produced.

The pectinolytic bacteria indigenous to the rumen are comprised of several species, including spiral organisms. The latter, recently described (5), are different from the spirochetes most commonly found in the rumen in being considerably larger. We have now also isolated some small rumen spirochetes utilizing pectin as sole substrate for growth. A description of these strains is briefly given below.

Methods for isolation and physiological tests for characterization of the spirochetes and analysis of the fermentation products were as previously described (5). The production, isolation, purification, and characterization of the pectinolytic enzymes were as reported by Wojciechowicz and Ziołecki (4).

Electron microscopy. Samples for negative staining were washed twice with 0.9% NaCl and suspended in distilled water. One drop of this suspension was mixed with 1 or 2 drops of 2% phosphotungstic acid adjusted to pH 7.2 with 5 N KOH. A drop of this mixture was placed on the support film side of the grid. After 30 s, excess fluid was removed with filter paper, and the preparation was thoroughly dried. The samples were examined in a Tesla BS 500 electron microscope operating at 60 kV.

Two strains of pectinolytic spirochetes were isolated at an interval of about 3 years from the rumen of a fistulated cow fed on hay and limited amounts of concentrates. Strain 692 was isolated from a 10^{-9} dilution on a pectin medium; strain 791 was isolated from a dilution of 10^{-8} on a similar medium containing 0.2% galacturonic acid instead of pectin. Morphologically the organisms resembled other small rumen spirochetes so far isolated and described (1, 6). The cells were helical with rounded and slightly tapered ends, 0.4 μm wide, and varied from 5 to 11 μm in length; the preponderance of the longer cells were strain 692, and most of the shorter ones were strain 791. The coils were fairly regular; their amplitude was about 0.9 μm, and the wavelength was about 2.1 μm. The number of coils varied from two to four and occasionally more. The cells were motile, exhibiting both a corkscrew-like and a lashing motion. Spherical bodies were formed during prolonged incubation, replacing the normal cells, as was also the case with other rumen spirochetes (1, 6). Electron micrographs showed ultrastructural features characteristic of the spirochetes (Fig. 1). The number of axial fibrils was eight; they were attached subterminally, four near each end of the cell. Strain 692 utilized only pectin. Strain 791, besides pectin, also fermented glucose, fructose, mannose, cellobiose, and sucrose. Growth of both strains on galacturonic acid was poor, even though strain 791 had been isolated on a galacturonic acid medium: the cells were elongated with a much less pronounced spiraling. Neither organism fermented D- or L-arabinose, D- or L-xylose, galactose, lactose, melezitose, rhamnose, raffinose, trehalose, starch, inulin, gum arabic, dextrin, xylan, glycerol, adonitol, mannitol, sorbitol, esculin, salicin, and lactate. Casein was not hydrolyzed, gelatin was not liquefied, indole was not produced, and nitrates were not reduced.

Pectin was fermented readily, yielding acetic and formic acids as the main fermentation products (Table 1). Strain 692 produced also small amounts of succinic acid, and strain 791 produced some lactic acid. A certain amount of methyl alcohol, derived from deesterification of pectin, was always found in the fermented medium. Propionic and butyric acids were not produced.

Growth on pectin was fairly rapid, though less rapid than that of the large spirochetes; the

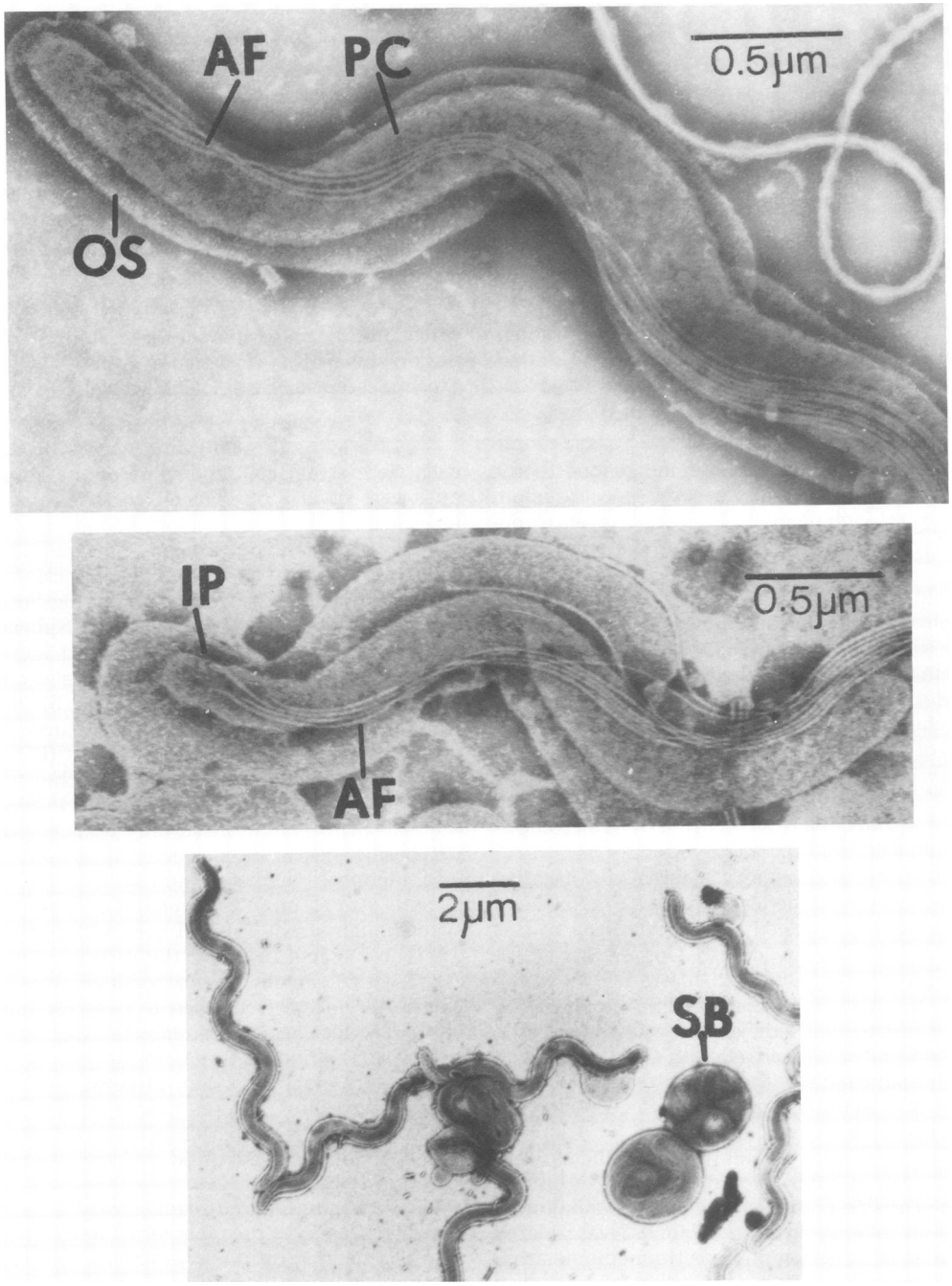

FIG. 1. *Electron micrographs showing general morphology and ultrastructure of the small pectinolytic rumen spirochete strain 692. The number of axial fibrils is eight in a 4-8-4 arrangement. Abbreviations used: AF, axial fibrils; IP, axial fibril insertion pore; OS, outer sheath; PC, protoplasmic cylinder; SB, spherical body.*

doubling time of strain 692 during exponential growth was about 85 min as compared to 60 min for the large spirochetes (5), and the lag phase was also considerably longer. The pectinolytic activity was also less than that of other pectin-decomposing rumen bacteria. The reduction in viscosity, estimated under standard conditions (7), of a 0.5% pectin solution incubated with the

cell-free culture fluid did not exceed 30%, as compared to over 70% in *Lachnospira multiparus*, *Bacteroides ruminicola*, and the large spirochetes (unpublished data).

The organisms produced an extracellular pectinolytic enzyme; the enzyme of strain 692 was isolated and characterized. The absorbance of the degradation products of polygalacturonate in the ultraviolet region and the thiobarbituric acid test (Fig. 2) showed that the enzyme acted by transelimination mechanism. Analysis by paper chromatography of the products of enzymic degradation of polygalacturonate suggested that the enzyme attacked the substrate chain in a random fashion from internal glycosidic bonds, giving mainly unsaturated digalacturonate as the end product (Fig. 3). Pectin was degraded less readily than polygalacturonate. On the basis of these results, the enzyme is classifiable as an endopolygalacturonate lyase (EC 4.2.2.2), which appears to be the principal pectinolytic enzyme of the pectin-metabolizing bacteria of the rumen so far examined: *B. ruminicola* (3), large spirochetes (4), and *L. multiparus* (M. Wojciechowicz, K. Heinrichova, and A. Ziołecki, J. Gen. Microbiol., in press).

A pectin pectylhydrolase (pectinesterase) (EC 3.1.1.11) was also produced by this organism, as shown by the cup-plate method of Reid (2), but no evidence could be found for the production of a polygalacturonase.

The general morphology of the cell and number of axial fibrils suggest that the organisms are treponemes. The results provide further evidence of the heterogeneity of the rumen spirochetal population and of the presence in the rumen of different physiological types occupying different ecological niches.

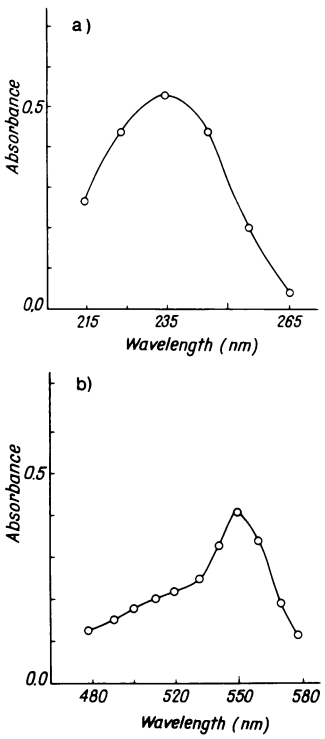

FIG. 2. *Ultraviolet absorbance (a) and thiobarbituric acid test (b) of degradation products of 0.5% polygalacturonate incubated with the enzyme after diethylaminoethyl-cellulose column chromatography and ultrafiltration in tris(hydroxymethyl)aminomethane-hydrochloride buffer at pH 8.0 and 30°C for 96 h.*

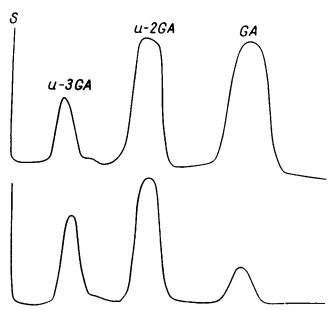

FIG. 3. *Densitometric illustration of spots on the chromatogram of degradation products as in Fig. 2 developed in pyridine-ethyl acetate-acetic acid-water (3:3:1:5) and visualized by alkaline silver nitrate reagent. Top line marked S is for standards of unsaturated trigalacturonate (u-3GA), unsaturated digalacturonate (u-2GA), and galacturonic acid (GA).*

TABLE 1. *Fermentation products of pectin by small pectinolytic rumen spirochetes*

Strain no.	Product (mmol/100 ml of fermented medium)[a]					Cell dry matter		Carbon recovery[b] (%)
	Formic acid	Acetic acid	Succinic acid	Lactic acid	CO_2[c]	mg	mmol of C[d]	
692	1.543	3.842	0.078	—	2.299	20.9	0.871	93.93
791	1.037	3.250	—	0.136	2.213	35.3	1.471	82.93

[a] Medium contained 0.55% of pectin, equivalent to about 440 mg of pectin galacturonic acid per 100 ml.
[b] Calculated relative to galacturonic acid C.
[c] Calculated as equimolar to acetic acid minus formic acid.
[d] Assuming 50% C in cell dry matter.

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