Sessions 5/6 Limitations of rumen fermentation

An enzymatic approach to cell wall structure

R.E. Hungate* and R.J. Stack
Department of Bacteriology
L.C. Greve and J.L. Labavitch
Department of Pomology
University of California, Davis,
California 95616, U.S.A.
*To whom correspondence should be addressed

Ruminococcus albus was incubated with isolated alfalfa cell wall material for 72 h in batch culture. Cellulose in the cell walls was digested to a somewhat greater extent (88%) than were the fermentable sugars of the hemicellulose fraction (62 – 76%). The digestibility of the total insoluble alfalfa cell wall, including lignin but not material solubilized during heat sterilization, was 66%. A cellulase, α-arabinosidase and xylanase were partially purified from the concentrated supernatant of *R. albus* cultures and a purified polygalacturonase was obtained from the fungus *Verticillium*. Of the enzymes tested, the most effective in digesting cell walls was the polygalacturonase. In addition, when mixed with α-arabinosidase, the amount digested exceeded the sum of the materials digested by the two enzymes separately.

Ruminococcus albus is vir 72 h met geïsoleerde lusernselwandmateriaal geïnkubeer in lotskultuur. Die sellulose in die selwande is tot 'n groter mate (88%) verteer as die fermenteerbare suikers van die hemisellulose gedeelte (62 – 76%). Die verteerbaarheid van die totale onoplosbare lusern selwande, insluitende lignien, maar nie materiaal wat tydens hittesterilisasie opgelos is nie, was 66%. 'n Sellulase, α-arabinosidase en xilanase is gedeeltelik gesuiwer vanuit die gekonsentreerde supernaat van die *R. albus* kulture en 'n gesuiwerde poligalakturonase is van die fungus *Verticillium* verkry. Van die ensieme wat getoets is, was die poligalakturonase die mees effektiewe verteerder van selwande. Verder het dit, wanneer gemeng met α-arabinosidase, 'n groter hoeveelheid selwandmateriaal verteer as die hoeveelheid materiaal verteer deur die twee ensieme afsonderlik.

Keywords: *Ruminococcus albus,* alfalfa cell walls, cellulose, hemicellulose, enzymic digestion

Introduction

The aim of the research is to provide more specific information on the chemical linkages in plant cell wall material. The procedure is (1) to determine which constituents of plant cell walls are digested by a culture of *Ruminococcus albus,* (2) to determine how much of the material in the walls can be digested by the mixture of extracellular enzymes of cultures grown on cellulose or cell walls, and (3) to separate and purify, as much as possible, the individual enzymes in the mixture to determine their digestive activity on cell walls singly and in various combinations with a view toward identifying the chemical linkages in the 3-dimensional wall structure.

Results and Discussion

Preliminary studies have been completed on cell walls prepared from the terminal 6 inches of vigorously growing alfalfa. The chemical methods of Albersheim's laboratory (Talmadge *et al.,* 1973) have been used to analyse quantitatively the kinds and amounts of sugars in the wall and in the products of digestion. The enzymes have been produced in cultures containing 0,15% pebble-milled cotton cellulose or 0,3% alfalfa cell walls (ACW). The composition of the wall material is shown in Table 1.

Table 1 Chemical composition of insoluble alfalfa cell wall material

	Sample		
Cell wall component	A	B	Mean
Neutral sugars[a]			
rhamnose	1,76%	1,92%	1,84%
arabinose	5,21	4,06	4,66
xylose	4,70	4,29	4,49
mannose	0,92	1,08	1,00
galactose	2,98	3,21	3,10
glucose	3,78	4,72	4,25
Uronic acids			21,4
Cellulose (sol. in 67% H_2SO_4)			25,6
Lignin (insol. in 67% H_2SO_4)			33,4

[a]Prepared by hydrolysis with 2N trifluoroacetic acid

The action of *R. albus* cultures on 18 mg of air-dried ACW in 6 ml of culture medium was estimated by analysing two sterilized tubes of culture medium, one of which was inoculated and incubated for 72 h at 39°C and the other incubated but not inoculated. The results are shown in Table 2. The heat sterilization in the autoclave solubilized 3,98 mg of the ACW sample. Since the soluble sugars had already been removed during the preparation of the ACW, the heat-solubilized material consisted exclusively of polymers of the various sugars. Of these only arabinose and the uronic acids were fermented to a significant extent, 58% and 25%, respectively, (not included in Table 2). Table 2 shows that the cellulose was digested to a somewhat greater extent, 88%, than were the fermentable sugars in the hemicellulose fraction, 62% to 76%. The digestibility of the total insoluble ACW, including lignin but not the heat-solubilized material, was 66%.

A cellulase and an α-arabinosidase were partially purified by gel filtration of the concentrated supernatant of *R. albus* cultures, the cellulase being obtained from cultures grown on cellulose and the α-arabinosidase from cultures grown on ACW. These enzymes, together with a purified polygalacturonase (PGase), obtained from the fungus *Verticillium,* were added singly and together to a sample of ACW that had not been heat sterilized. In addition, a

Table 2 Components of insoluble alfalfa cell walls fermented by a culture of *Ruminococcus albus*

Component	Unfermented culture	Fermented culture	Percentage fermented
Solubilized by the heat sterilization	3,98 mg	3,11 mg	22%
Hemicellulose (sol. in 2% TFA)			
glucose	0,488	0,130	73
galactose	0,357	0,135	62
mannose	0,150	0,145	nil
xylose	1,003	0,272	73
arabinose	0,236	0,085	64
rhamnose	0,124	0,132	nil
uronic acids	1,240	0,278	76
Cellulose (insol. in 2% TFA)	6,986	0,372	88

xylanase preparation containing also PGase activity was obtained from *R. albus* and was tested along with the mixture of the other three enzymes. The results are shown in Table 3.

Table 3 Digestion of alfalfa cell walls[a] by purified enzymes obtained from *Ruminococcus albus* and a fungus

Enzymes[b]	Sugar digested	Uronic acids digested	Total
Polygalacturonase (PGase)	0,534 mg	1,172 mg	1,706 mg
α-Arabinosidase	0,035	0,093	0,128
Cellulase	0,123	0,654	0,777
PGase, α-arabinosidase	0,982	2,261	3,243
PGase, cellulase	0,929	1,361	2,290
Cellulase, α-arabinosidase	0,455	0,588	1,043
PGase, α-arabinosidase cellulase, xylanase	1,854	1,670	3,524

[a] Weight of the substrate ACW was 20 mg, air dry.

[b] Cellulase, α-arabinosidase and xylanase were purified from concentrated supernatants of *R. albus* cultures. Polygalacturonase was obtained from the fungus *Verticillium*.

Of the enzymes tested the most effective in digesting the ACW was the PGase. In addition, when mixed with α-arabinosidase, the amount digested exceeded the sum of the materials digested by the two enzymes separately. This synergism was shown also by the mixture of PGase with cellulase, although to a lesser extent. The purified cellulase, only one of several that are formed by *R. albus,* was relatively ineffective in digesting ACW, when compared with the results obtained by the total enzymes in the culture (Table 2). This may account in part for the relatively smaller amount of xylose digested.

These preliminary results indicate that additional enzymes must be purified and added to the test mixture to achieve a digestion by known enzymes equal to that shown by the culture supernatant.

References

TALMADGE, K.W., KEEGSTRA, K., BAUER W.D. & ALBERSHEIM, P. 1973. The structure of plant cell walls. I. The macromolecular components of the walls of suspension-cultured Sycamore cells with a detailed analysis of the pectic polysaccharides. *Pl. Physiol.* 51, 158 – 173.