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Relationship Between Cellulolytic Activity and Adhesion to Cellulose in *Ruminococcus albus*

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Bacterial adhesion to cellulose was measured for 13 cellulolytic and 10 non-cellulolytic, xylan-utilizing strains of the ruminal bacterium *Ruminococcus albus*. Radiolabelled bacteria adhering to Whatman CF11 cellulose powder were determined. Adhesion of the cellulolytic strains ranged from 0 to 49% of the added bacteria. Of the non-cellulolytic strains, 9 showed <1% adhesion, while one strain gave 5% adhesion. For the cellulolytic strains filter paper solubilization ranged from 24 to 100%, while solubilization of CF11 cellulose varied from 0 to 20%. Both cellulolytic and non-cellulolytic strains produced carboxymethylcellulase (CMCase) activity. SDS-PAGE of cell extracts followed by incubation with a gel overlay containing CMC or xylan produced a zymogram of hydrolytic enzyme activity. The cellulolytic strains showed a number of bands of CMCase and xylanase activity. Non-cellulolytic strains possessed fewer bands of activity towards both CMC and xylan. Certain of the enzymes appeared to possess both CMCase and xylanase activity. Bacterial cell surface hydrophobicity was also measured, but no correlation was found between hydrophobicity and adhesion to cellulose.

INTRODUCTION

In a continuous flow ecosystem such as the rumen, bacteria which attach to and colonize solid digesta have a distinct advantage in a competitive environment. The solid digesta are retained in the rumen for longer than the liquid fraction of the rumen contents, and organisms attached to the solid particles are therefore able to maintain their numbers at a lower growth rate. In addition, adherent fibre-digesting bacteria can bring cell-bound enzymes into contact with their substrate, thereby ensuring effective digestion and enabling the degradation products to be immediately available to the bacteria concerned.

Adhesion may be especially important where the digestion of a complex substrate such as a plant cell wall or cellulose is involved, since a number of enzymes must act in combination. When bacteria adhere to the substrate, a complex of enzymes located on the bacterial cell surface can be presented to the substrate in an optimal configuration. Such a complex, consisting of both enzymes and specific adhesion proteins, known as a 'cellulosome' has been proposed for the cellulolytic (non-ruminal) organism *Clostridium thermocellum* (Bayer *et al.*, 1983; Lamed *et al.*, 1983; Bayer *et al.*, 1985). Such adhesion proteins or 'adhesins' could impart a degree of specificity to the adhesion reaction. For instance, it is known that certain rumen bacteria adhere preferentially to particular types of plant cell wall (Latham *et al.*, 1978). In contrast, less refractory types of cell wall can be degraded by soluble enzymes without the need for adhesion (Akin *et al.*, 1974; Akin, 1980).

Ruminococcus albus is one of the most important cellulolytic rumen bacteria, and has been shown to adhere to cellulose fibres (Patterson *et al.*, 1975; Minato & Suto, 1978) by a mechanism which is inhibited by various soluble cellulosic derivatives (Minato & Suto, 1978). However, it

Abbreviation: CMC, carboxymethyl cellulose.

has not been determined to what extent adhesion is a prerequisite for cellulolysis by this organism and whether adhesion is mediated by particular cellulolytic enzymes. The existence of a group of non-cellulolytic strains of *R. albus* isolated in this laboratory (van der Toorn & van Gylswyk, 1985) has made possible a comparative study of the adhesion properties and enzyme profiles of cellulolytic and non-cellulolytic strains.

METHODS

Bacteria. Ruminococcus albus cellulolytic strains X10C33, X10C56, X3D54, X6D60 and X12D62, as well as the non-cellulolytic strains X2A57, X7A62, X5C54, X10C62, X9C63, X6D34, X6D38, X9D54, X8D60 and X6E39, were obtained from the rumens of sheep fed maize straw diets (van der Toorn & van Gylswyk, 1985). These strains were all isolated on the basis of their ability to degrade xylan, and were maintained on xylan agar slants.

Of the other cellulolytic strains of *R. albus*, 22.08.6A and 21.09.6E were described by Kistner & Gouws (1964), Ce54 and Ce63 by Shane *et al.* (1969) and strain 30 by van Gylswyk and Roché (1970). Strain AcTF10 was isolated by Henning (1979) and was extensively characterized by Morris & van Gylswyk (1980), who also gave microscopic evidence of its attachment to plant cell walls. Strains 7 and 20 were a gift from M. P. Bryant, University of Illinois, Urbana-Champaign, USA, and were positive when tested for visual disintegration of filter paper (Bryant *et al.*, 1958). All these strains were maintained on cellobiose agar slants. *Bacteroides succinogenes* S-85 and *Ruminococcus flavefaciens* FD-1 were gifts from M. P. Bryant.

Culture techniques. Procedures for obtaining and maintaining strictly anaerobic conditions were as described by van Gylswyk & Hoffmann (1970).

Culture conditions. For measurement of adhesion and determination of hydrophobicity, the bacteria were grown in a liquid medium based on medium 10 of Caldwell & Bryant (1966) with 0·3% cellobiose as carbon source and a gas phase containing 30% (v/v) CO_2, 65% (v/v) N_2 and 5% (v/v) H_2. For other purposes a medium supplemented with 30 or 40% (v/v) rumen fluid (clarified by centrifugation at 13000 *g* for 1 h) was used, with either cellobiose or cellulose as carbon source, and 95% (v/v) CO_2/5% (v/v) H_2 in the gas phase.

Cellulose degradation. The ability of the various bacterial strains to solubilize cellulose was determined as described by Morris & van Gylswyk (1980) with minor modifications. Inocula were grown on cellobiose agar slants. Cellulose substrates (Whatman no. 6 filter paper, ball-milled and freeze-dried, or Whatman CF11 cellulose powder) were used at a 1% (w/v) concentration. Bacteria were inoculated into 10 ml culture medium containing the cellulose, and incubated at 39 °C for 7 d, after which the remaining cellulose was washed on a sintered glass filter, dried, and determined gravimetrically.

Assay for carboxymethylcellulase (CMCase) production. CMCase activity produced by colonies growing on cellobiose agar plates was detected using the Congo red plate technique of Teather & Wood (1982).

Measurement of adhesion to cellulose. Cells were radiolabelled by the addition of an aqueous solution (37 MBq ml^{-1}) of sodium [1-^{14}C]acetate (Amersham, 2 GBq $mmol^{-1}$) to the culture medium to give a final concentration of 37 kBq ml^{-1}. Bacteria were grown to stationary phase in the presence of the radionuclide, then harvested and washed three times with a mineral buffer containing 1·3 mM-KH_2PO_4, 1·7 mM-K_2HPO_4, 3·4 mM-$(NH_4)_2SO_4$, 0·76 mM-NaCl, 0·38 mM-$MgSO_4$ and 0·41 mM-$CaCl_2$, pH 7·0. These salts were used at the same concentration as supplied in the culture medium. The cells were harvested and washed aerobically except where specified.

The washed cells were resuspended to give an optical density at 660 nm of 2·0 (0·54 mg dry weight ml^{-1}), determined by appropriate dilution. The cell suspension was then added to a glass scintillation vial containing 100 mg cellulose (Whatman CF11) which had been previously washed twice in distilled water (20 ml) and once in the mineral buffer (20 ml) to remove fines. Excess liquid was removed each time by aspiration after leaving the cellulose to settle for 5 min.

The cell suspension was gently shaken with the cellulose for 30 min at room temperature, after which the mixture was diluted by addition of 20 ml mineral buffer. After leaving the cellulose to settle for 5 min the supernatant was removed by aspiration. The cellulose was washed twice more with buffer in the same way. Scintillation fluid (10 ml) was then added to the washed cellulose, and radioactivity was measured on a scintillation counter. Determinations were done in triplicate, and control vials containing no cellulose were included to measure adhesion to the vial itself and to allow for any clumps of bacteria which might settle from suspension although they were not in fact adherent.

To examine the influence of anaerobic conditions on adhesion, cells were harvested, washed and mixed with cellulose under an atmosphere of 30% CO_2/65% N_2/5% H_2 in an anaerobic cabinet. The mineral buffer was made up with oxygen-free water, and pre-equilibrated with the anaerobic gas mixture before adjustment of pH. In some instances 1 mM-dithiothreitol was added to the buffer. Parallel experiments were done under aerobic conditions for comparison.

Solubilization of R. albus cells. The cells of *R. albus* were resistant to the action of lysozyme, but could readily be solubilized by mutanolysin (Sigma). Bacteria grown in rumen fluid medium with 0·5% cellobiose were harvested by centrifugation, washed twice in mineral buffer, and resuspended in 20 mM-Tris/HCl, 1 mM-$MgCl_2$, pH 6·8, to

an optical density of 6·5 at 660 nm (1·76 mg dry weight ml^{-1}), determined by appropriate dilution. Mutanolysin was added to a concentration of 50 units ml^{-1} and the mixture was incubated overnight at 39 °C. The optical density of the cell suspension decreased by about 90% following incubation. Remaining cells and cell debris were removed by centrifugation, and the supernatant was stored frozen until required for SDS-PAGE analysis.

SDS-PAGE. This was done according to Laemmli (1970) using 7·5% (w/v) polyacrylamide gels. Samples were preincubated at 39 °C for 1 h in 2% (w/v) SDS, 5% (w/v) 2-mercaptoethanol before loading on to the gel. The samples were not boiled since it was desired to retain enzyme activity. M_r markers were myosin (M_r 205000), β-galactosidase (M_r 116000), phosphorylase-b (M_r 97400), BSA (M_r 66000), ovalbumin (M_r 45000) and carbonic anhydrase (M_r 29000), from Sigma. Gels were silver-stained according to the method of Oakley *et al.* (1980). M_r values were estimated according to Plikaytis *et al.* (1986).

Gel replica technique for localization of CMCase or xylanase activity. CMCase and xylanase activities were detected in polyacrylamide gels by modification of the method of Beguin (1983). Following SDS-PAGE, the gel was shaken in a solution of 2·5% (v/v) Triton X-100 for 30 min. The SDS-PAGE gel was then sandwiched between polyacrylamide gels (7·5% polyacrylamide) which contained 0·1% CMC (CMC 7LP, Hercules Inc., Wilmington, Del., USA) or 0·1% xylan (Fluka) and 0·2 M-sodium phosphate buffer, pH 6·8. The gels were left together at room temperature overnight, after which the substrate-containing gels were stained with Congo red (1 mg ml^{-1}) for 1 h, followed by washing in 1 M-NaCl until bands became visible.

Determination of bacterial cell surface hydrophobicity. Hydrophobicity was measured by the ability of the cells to bind to hexadecane (Westergren & Olsson, 1983) as well as by the 'salting out' procedure of Lindahl *et al.* (1981), with ammonium sulphate concentrations ranging from 0·1 M to 2·5 M. Both procedures were carried out in the mineral buffer described earlier.

RESULTS

Solubilization of cellulose

The majority of strains of *R. albus* used in this study were originally designated as cellulolytic or non-cellulolytic by measurement of acid production in a culture medium containing filter

Table 1. *Cellulose degradation and adhesion to Whatman CF11 cellulose powder by strains of R. albus*

Strain	Cellulose solubilized (%)		Bacteria adhering to Whatman CF11 cellulose powder (% of cells added)
	Whatman no. 6 filter paper	Whatman CF11 cellulose powder	
Cellulolytic			
X10C33	82	6	33·5
X10C56	99	1	18·0
X6D60	48	0	13·8
X12D62	79	0	13·8
X3D54	100	3	19·0
21.09.6E	99	20	14·6
22.08.6A	99	20	20·0
Ce54	24	1	0
Ce63	67	23	27·1
7	99	7	12·1
20	99	2	49·0
30	100	7	15·9
AcTF10	100	1	3·4
Non-cellulolytic			
X9C63	0	0	0·3
X5C54	0	0	0·6
X6D38	1	3	0·4
X10C62	0	1	0·2
X6D34	0	0	0·8
X8D60	0	0	0·5
X9D54	0	0	0
X7A62	0	0	4·6
X2A57	1	0	0·3
X6E39	0	3	0·4
B. succinogenes S-85	52	54	
R. flavefaciens FD-1	54	9	

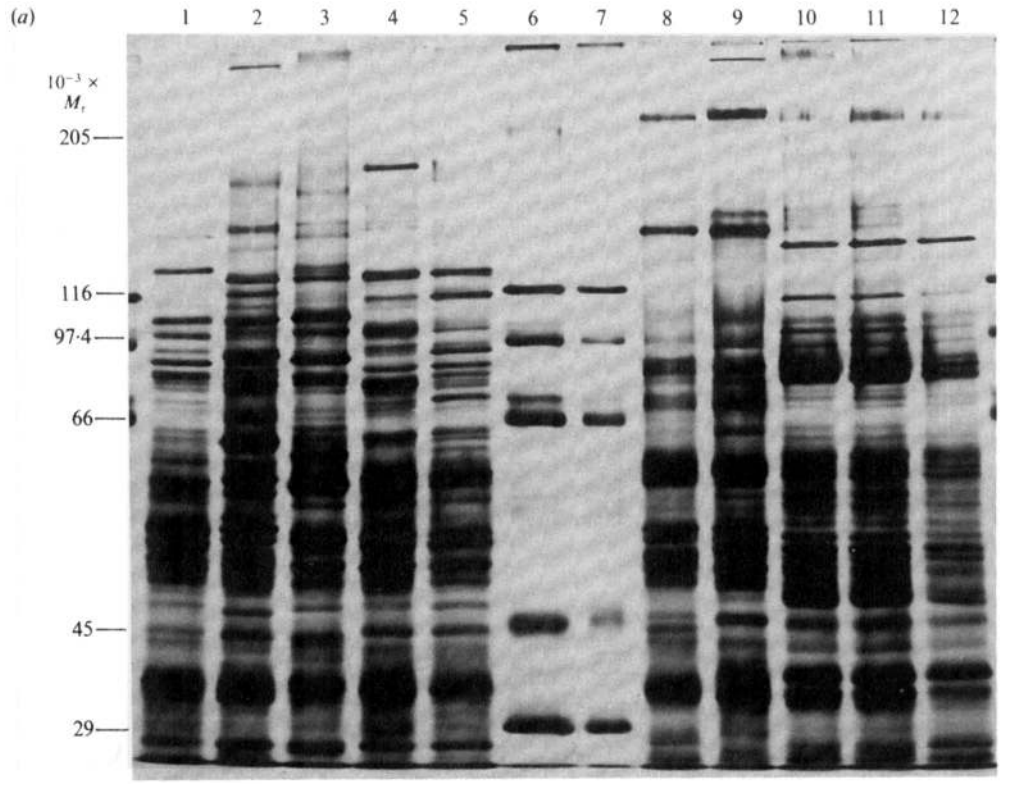
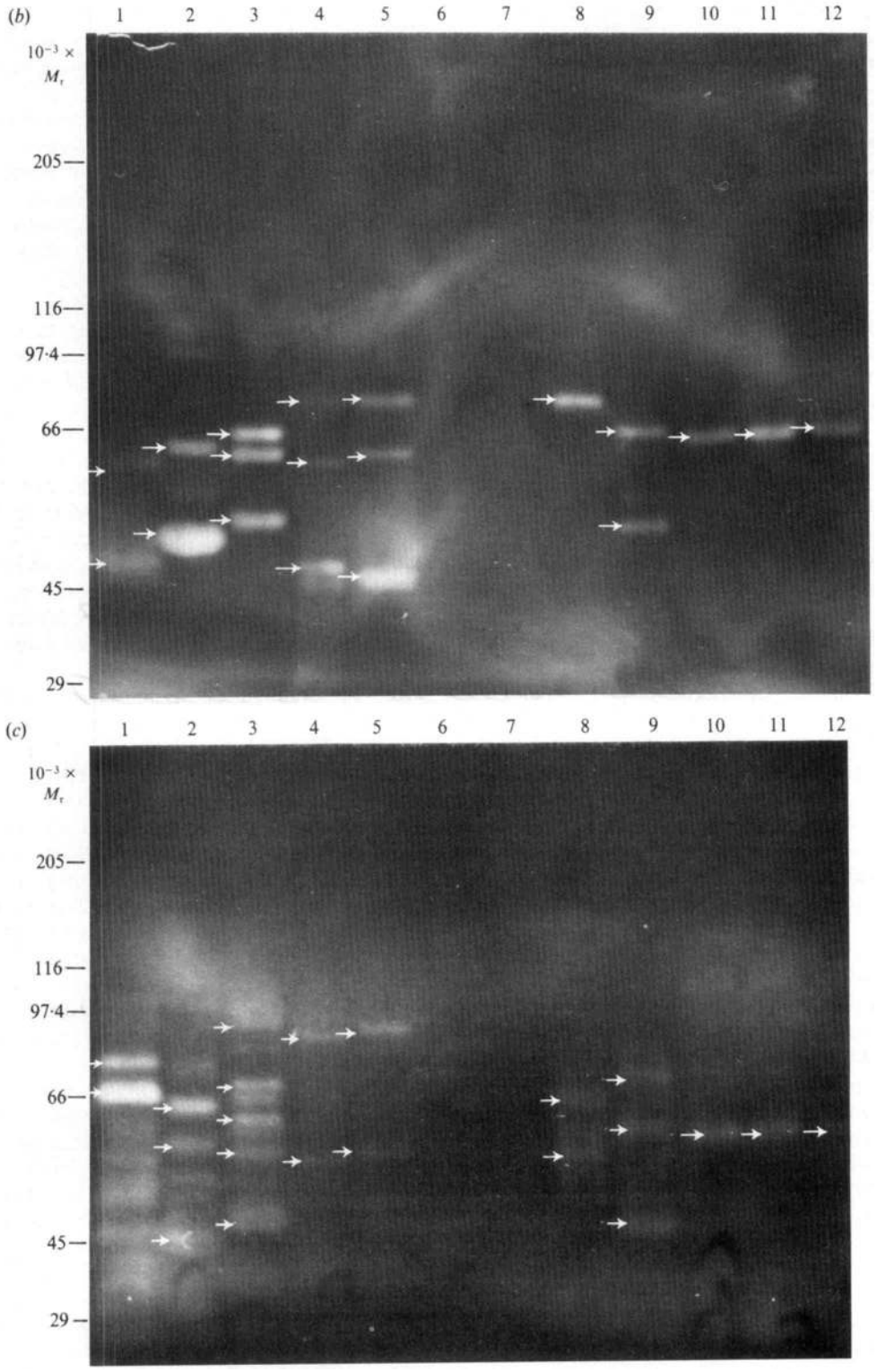


Fig. 1 (continued on facing page). SDS-PAGE of cells of *R. albus* digested with mutanolysin, showing total protein profiles revealed by silver staining (*a*), CMCase activity (*b*) and xylanase activity (*c*). Bands indicated by small, white arrows in (*b*) and (*c*) correspond to M_r values given in Table 2. Lanes 1–5, cellulolytic strains: 1, X10C33; 2, 20; 3, X6D60; 4, X3D54; 5, X10C56. Lanes 6 and 7, M_r markers. Lanes 8–12, non-cellulolytic strains: 8, X9D54; 9, X7A62; 10, X2A57; 11, X6D34; 12, X8D60.

Table 2. *Estimated M_r values of CMCase and xylanase activities from SDS-PAGE*

	$10^{-3} \times M_r$	
Strain	CMCase	Xylanase
Cellulolytic		
X10C33	56, 42	72, 66
20	58, 45	64, 57, 44
X6D60	60, 57, 48	80, 67, 60, 56, 47
X3D54	66, 55, 42	76, 54
X10C56	66, 56, 41	79, 56
Non-cellulolytic		
X9D54	65	65, 56
X7A62	59, 46	69, 59, 45
X2A57	59	59
X6D34	59	59
X8D60	59	59


(b)
1 2 3 4 5 6 7 8 9 10 11 12
$10^{-3} \times M_r$
205
116
97·4
66
45
29
(c)
1 2 3 4 5 6 7 8 9 10 11 12
$10^{-3} \times M_r$
205
116
97·4
66
45
29

paper cellulose as the sole carbon source. However, for most of the strains nothing was known of the extent of cellulose degradation of which they were capable, nor of their activity on other forms of cellulose such as Whatman CF11, which was used in subsequent adhesion assays. Whatman CF11 is a crystalline cellulose derived from cotton linters.

Measurements were made of the total extent of cellulose solubilization over an incubation period of one week (Table 1). Figures obtained for *B. succinogenes* and *R. flavefaciens* are included for comparison. The strains originally designated as non-cellulolytic did not degrade either filter paper cellulose or CF11 cellulose powder to any significant extent. Among the cellulolytic strains there was considerable variation in the ability to degrade both forms of cellulose, although the CF11 cellulose was only poorly attacked by all strains of *R. albus*.

Production of CMCase

Although the non-cellulolytic strains of *R. albus* did not degrade insoluble cellulose, they all produced significant amounts of CMCase, as demonstrated by the production of colourless zones when colonies grown on agar plates were overlaid with agar containing CMC (data not shown).

Adhesion of R. albus to cellulose

Bacteria were radiolabelled for adhesion assays by inclusion of sodium [1-^{14}C]acetate in the culture medium. In preliminary experiments with [*methyl*-1′,2′-^{3}H]thymidine to label the cells (Morris & McBride, 1984) no uptake of radioactivity was obtained. Sodium acetate was subsequently selected because Allison & Bryant (1963) had demonstrated its incorporation into cells of *R. flavefaciens*. Incorporation of radioactivity ranged from 1 to 3 kBq per mg dry weight of cells. The radionuclide was judged to be stably incorporated into the bacteria since after the first wash almost no radioactivity was found in the supernatant following each subsequent centrifugation step.

Whatman CF11 cellulose powder was chosen for the measurement of adhesion since it settled quickly from suspension and could therefore easily be separated from non-adherent bacteria. Microscopic examination revealed bacteria adhering to the cellulose, with few free cells, demonstrating that the recovered radioactivity was not due to clumps of bacteria settling out with the cellulose. This was confirmed by the fact that radioactivity in control vials (no added cellulose) was low. Preliminary experiments indicated that most of the bacterial adhesion occurred within 5 min of adding a cell suspension to the cellulose and that little further change occurred after 2 h incubation. However, 30 min was taken as a standard incubation period. Adhesion assays were routinely carried out under aerobic conditions as this facilitated experimental procedures. This could be justified since adhesion was not found to be significantly affected by anaerobic conditions, either with or without addition of 1 mM-dithiothreitol. The optimum temperature for adhesion was found to be around 30 °C. However, there was a fairly broad optimum, with little difference between adhesion at 25 °C (approximate ambient temperature) and at 39 °C. Experiments were therefore conducted at room temperature.

Among the non-cellulolytic strains only X7A62 showed significant adhesion to cellulose (Table 1). Among the cellulolytic strains adhesion ranged from 0 to 49%. However, only two strains (Ce54 and AcTF10) showed less than 10% adhesion. Within the group of cellulolytic strains there was apparently no correlation between the extent of attachment to CF11 cellulose and the ability to degrade it; for instance strain 20 showed 49% adhesion but no significant solubilization, while strain 21.09.6E showed only 15% adhesion but solubilized 20% of the substrate. However, it was of interest that strain Ce54, which did not adhere at all, was also the cellulolytic strain with the least activity on filter paper cellulose.

SDS-PAGE of cell extracts

Cells of strains of *R. albus* were digested with mutanolysin and the solubilized material was fractionated by SDS-PAGE. Zymograms of CMCase and xylanase activity were obtained by the gel overlay technique. Little protein was visible in SDS-PAGE gels stained with silver nitrate after incubation with substrate-containing gels, suggesting that most of the enzyme

Table 3. *Cell surface hydrophobicity of R. albus determined by aggregation in ammonium sulphate*

Results are expressed as the lowest molarity of $(NH_4)_2SO_4$ which gave visible clumping of bacteria when visualized against a dark background and compared with a control [no $(NH_4)_2SO_4$].

Strain	Hydrophobicity	Strain	Hydrophobicity
Cellulolytic		Non-cellulolytic	
X10C33	ND	X9C63	2·0
X10C56	<0·1	X5C54	2·0
X6D60	1·0	X6D38	1·0
X12D62	<0·1	X10C62	2·0
X3D54	0·5	X6D34	1·0
21.09.6E	1·0	X8D60	0·5
22.08.6A	2·0	X9D54	1·5
Ce54	2·0	X7A62	0·5
Ce63	1·0	X2A57	1·5
7	1·5	X6E39	ND
20	1·0		
30	2·5		
AcTF10	ND		

ND, Not determined.

activity had diffused into the gel overlay. Fig. 1 shows both the total protein profiles as visualized by silver staining and the location of enzyme activity for five cellulolytic and five non-cellulolytic strains selected at random. The estimated M_r values of some major bands possessing enzyme activity are given in Table 2. The cellulolytic strains all possessed a number of bands with CMCase and xylanase activity, but there were marked differences between strains both in the location of these bands and in the total protein profiles (Fig. 1*a*). Cellulolytic strains not shown in Fig. 1 also varied considerably both in their protein profiles and in the position of bands with enzyme activity. Certain strains did however show similarities. Strains X3D54 and X10C56 were similar (Fig. 1) and there was also some correspondence between 21.09.6E and 22.08.6A as well as between 30 and AcTF10.

The non-cellulolytic strains had fewer CMCase and xylanase bands, and there was less variation between strains. Of the strains shown in Fig. 1, X2A57, X6D34 and X8D60 had very similar enzyme and protein profiles. These strains appeared to be part of a larger group which included X9C63, X5C54 and X6D38. Strain X7A62 (the only non-cellulolytic strain to show some adhesion) had a somewhat different protein profile from the other strains in this group and possessed an additional band of enzyme activity (Fig. 1). The remaining non-cellulolytic strains (X9D54, X6E39 and X10C62) formed a second group with similar enzyme and protein profiles.

For the non-cellulolytic strains there was good correlation between the positions of bands with CMCase activity and bands with xylanase activity, suggesting that the same enzyme had activity against both substrates. In contrast, for the cellulolytic strains several of the CMCase bands did not correspond with zones of xylanase activity, while a variety of xylanase bands did not have counterparts in the CMC gel. Enzyme activities in mutanolysin digests stored at −20 °C were stable for a few weeks, but some changes in band patterns were seen after storage for longer periods.

Cell surface hydrophobicity

All strains of *R. albus* were completely hydrophilic by the hexadecane binding assay as practically all the cells remained in the aqueous layer. The ammonium sulphate 'salting out' procedure did reveal some differences between strains (Table 3). However, there was no apparent correlation between the ammonium sulphate concentration required for the clumping reaction and the ability of the strains to adhere to cellulose (Table 1).

DISCUSSION

The development of an assay using radiolabelled cells to measure adhesion to cellulose has enabled an assessment to be made of the relative abilities of strains of *R. albus* to adhere to cellulose. Although Latham *et al.* (1979) refer to the use of ^{14}C-labelled bacteria to measure adhesion of rumen bacteria to plant stems, this is believed to be the first complete account of such an assay.

The results demonstrate a relationship between the ability to adhere to CF11 cellulose and the ability to degrade filter paper. However, there was no correlation between adhesion and solubilization of CF11 cellulose, since many of the adherent strains were unable to degrade this substrate. Thus it seems that adhesion is not necessarily followed by cellulolysis. It appears from the results obtained here that strains which cannot adhere produce only limited cellulolysis. In contrast Kauri & Kushner (1985) found that for a variety of bacteria, degradation of cellulose did not depend on cell–fibre contact. Unfortunately none of the rumen bacteria were included in their study. As pointed out by Wood & Wilson (1984), cell-free enzyme extracts are often not as effective as the whole cells in degrading cellulose, demonstrating that some essential factor present on the bacterium is missing in the cell-free extract.

It is not clear at present whether the non-adherent strains of *R. albus* are unable to bind to cellulose because of the lack of specific 'adhesion factor' or 'affinity factor' as proposed by Leatherwood (1969), as a result of which cell-associated enzymes cannot come into contact with their substrate, or alternatively whether the lack of particular cellulolytic enzymes capable of binding to the insoluble substrate in itself prevents adherence. If adhesion is mediated by cellulases, then endocellulases, β-glucosidases (cellobiases) or exocellulases (if present) could be responsible. Wood & Wilson (1984) found no evidence for exocellulase production by *R. albus*. In the work described in this paper the possible role of endocellulases in adhesion was studied. Examination of the endocellulase (CMCase) activity produced by adherent (cellulolytic) and non-adherent (non-cellulolytic) strains grown on agar plates and overlaid with agar containing CMC revealed production of CMCases by all strains. However, in the context of adhesion it was important to know whether such enzymes were bound to the cell surface or whether they were secreted into the culture medium. Washed bacterial cells were therefore solubilized by digestion with mutanolysin. It is presumed that cellulase and xylanase activities released in this way are located on the cell surface, since it is unlikely that polysaccharide-degrading enzymes would have an intracellular location. The solubilized material was fractionated by SDS-PAGE and enzyme activity was located in the gels using a zymogram technique. All the cellulolytic and non-cellulolytic strains tested possessed cell-associated CMCases and xylanases, although the non-cellulolytic strains clearly had fewer bands of activity than the cellulolytic strains. The fact that the non-cellulolytic (and non-adherent) strains possess cell-associated CMCase activity does not however rule out the possibility that this activity could mediate adherence. It has recently been shown for fungal cellulases (Hayashida & Mo, 1986; Klyosov *et al.*, 1986) that while a variety of endoglucanases are able to degrade soluble cellulosic derivatives, not all such enzymes have the ability to bind to insoluble cellulose. Such binding ability might constitute the 'C_1' activity supposedly required for hydrolysis of highly ordered celluloses (Reese *et al.*, 1950).

Several workers have identified a large cellulase complex on the surface of *R. albus* (Stack & Hungate, 1984, 1985; Wood *et al.*, 1982). Such a complex might correspond to the 'cellulosome' of *C. thermocellum* (Bayer *et al.*, 1985) and could be expected to contain the full complement of enzyme and adhesive activities. Any alteration in one component of such an aggregate might affect the total cellulolytic activity, as well as affecting the adhesive ability of the complex. Wood *et al.* (1982) found that much of the high-M_r complex was removed from the cell surface by washing with a high-ionic-strength buffer. We have also found some enzyme released in this way. To minimize enzyme release, the cells were washed briefly (twice only) in a fairly low-ionic-strength buffer before digestion with mutanolysin. Zymograms obtained after mutanolysin digestion indicated that considerable enzyme activity was retained on the cell surface, and the range of M_r values corresponds well with those produced by disaggregation of the high-M_r complex (Wood *et al.*, 1982).

The multiplicity of enzyme activities revealed in the zymograms could be partially the result of incomplete denaturation, since the samples were not pre-boiled. For this reason, and because

the M_r markers are not visible on zymograms, the M_r values given in Table 2 may be inaccurate. However, each organism produced a characteristic zymogram in repeated experiments. The enzyme activities seen do not necessarily represent the full complement of endoglucanases and xylanases, since only those activities which are renaturable after treatment with SDS will show activity on the zymogram.

Marked differences between strains were apparent in the SDS-PAGE protein profiles (Fig. 1 *a*) and in the zymograms (Fig. 1 *b, c*). All the strains produced fermentation end products characteristic of *R. albus*, but it is possible that they may not be closely related. Jarvis (1967) found considerable serological diversity amongst organisms of the genus *Ruminococcus*.

Anaerobic conditions did not appear to stimulate adhesion of *R. albus*. This was of interest since Smith *et al.* (1973) previously demonstrated that cellulolysis in *R. albus* was partially inhibited by oxygen. They interpreted this as a complete inhibition of one or more, but not all, of the enzymes. The lack of oxygen sensitivity in adhesion to cellulose cannot however be taken as a general rule since Kopecny *et al.* (1983) found that adhesion of mixed rumen micro-organisms was markedly stimulated by addition of dithiothreitol.

In contrast with many other biological systems (Edebo *et al.*, 1980), no correlation was found between bacterial adhesion and the possession of a hydrophobic cell surface. This was perhaps not surprising since it is not to be expected that hydrophobic interactions would play a role in adhesion to a hydrophilic substrate such as cellulose. Jones and Isaacson (1983) have stressed the need to use several procedures in evaluating adhesin hydrophobicity. In this instance all strains were hydrophilic by the hexadecane assay, but differences between strains could be seen by using the ammonium sulphate technique. However, these differences may be indicative of variations in surface components unrelated to those which mediate adhesion. In this context Fives-Taylor & Thompson (1985) found that while the majority of non-adherent mutants of *Streptococcus sanguis* had a decreased hydrophobicity, some in fact showed an increase in hydrophobicity.

Work is currently in progress to further characterize the cell surfaces of these bacteria, with the aim of identifying specific molecules responsible for adhesion.

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