by colonic bacteria, the original set of polysaccharides may be replaced by a different mixture of polysaccharides and oligomers, and the properties of this new mixture (e.g., the ability to bind ions or toxic substances) may be very different from the properties of the original mixture. This possibility should be taken into account by workers who are investigating properties of dietary fiber components which may have physiological importance in the human intestinal tract.

Finally, the ability of the organisms to produce the enzymes necessary to enable them to switch to a new carbohydrate source was significantly decreased when generation times were long. If this is true in general for colon bacteria, bacteria which are capable of utilizing either dietary polysaccharides or polysaccharides in host secretions may be more likely to utilize polysaccharides which are always available, i.e., the polysaccharides in host secretions, rather than polysaccharides from the diet which are much more variable with respect to availability and digestibility. The effect of diet, and especially of the digestibility of dietary components by colonic bacteria, on the extent to which these bacteria degrade host mucins and other host secretions needs to be investigated since bacterial degradation of these substances might have some impact on the health of the host.

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# Enzymatic Degradation of the Plant Cell Wall by a *Bacteroides* of Human Fecal Origin

Jane Dekker*[1] and James K. Palmer

The isolated cell wall of peanuts contained about 20% pectic substances, 20% cellulose, and 30% hemicelluloses. An inoculum of human feces was able to ferment the cell wall, and eight organisms capable of fermenting this substrate were subsequently isolated. One of these, identified as a *Bacteroides*, was selected for a study of the enzymatic mechanism of cell wall degradation. The cell wall degrading activity was predominantly intracellular and present at low levels in bacteria grown on glucose or xylose. The intracellular enzyme activity increased ~6-fold in bacteria grown on the peanut cell wall, and the increased activity was present within 4 h of inoculation into the cell wall medium. The intracellular enzyme fraction from bacteria grown on the cell wall liberated ~11% of the cell wall sugars during an 18-h incubation. Uronic acid and xylose were the predominant products. These results with a prototype dietary fiber indicate the potential for fiber degradation in the human colon, which could alter the properties of the fiber.

The physiological effects of dietary fiber are difficult to evaluate because of the complexity and variability of fiber composition. Many of the therapeutic properties which are attributed to dietary fiber pertain to its function in the colon. It has been postulated that hydrated fiber may cause a decrease in transit time of materials through the colon, may dilute potentially harmful compounds, or may actually bind certain classes of compounds, most notably bile salts and acids (Mitchell and Eastwood, 1976). Current research is attempting to relate the chemical structure and the physical properties of dietary fiber to its physiological function. There is evidence, however, which suggests that the chemical structure of dietary fiber may be modified during transit through the colon. The saccharolytic microorganisms which populate the colon may attack some polysaccharide components of dietary fiber. The evidence which supports such an alteration of dietary fiber

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has been reviewed (Salyers et al., 1979) and includes nutritional balance studies in which dietary cellulose and hemicellulose were incompletely recovered in the feces (Southgate and Durnin, 1970). In addition, studies surveying the capability of colon microflora to ferment model cell wall polysaccharides revealed that many strains were capable of some polysaccharide utilization (Salyers et al., 1977b,c). Subsequent study of several strains of *Bacteroides* revealed that these strains degraded selected polysaccharide substrates by means of enzyme induction (Salyers et al., 1978).

In view of this demonstrated capacity for model cell wall polysaccharide degradation, the present study sought to establish the capability of a bacterium of human origin to ferment the insoluble and intact plant cell wall. For this investigation the cell wall of peanuts was employed as a prototype dietary fiber.

## MATERIALS AND METHODS

**Isolation of the Cell Wall of Peanuts.** Oven-dried, commercially roasted peanuts were coarsely chopped and Soxhlet-extracted for 8 h with diethyl ether. The defatted peanuts were finely ground and subjected to an enzymatic digestion procedure, designed to approximate the human digestive process. This procedure, modified from Hellendoorn et al. (1975), involved a 9-h incubation in 0.1 N HCl with pepsin, followed by a 9-h incubation at pH 6.8 with pancreatin. The resultant residue was washed 3 times with water and 3 times with acetone. This peanut residue was determined to be essentially starch free. For the purpose of this study, the peanut residue was defined as the cell wall.

The cell wall was maintained at 7.4% moisture by storage in a closed chamber over a saturated solution of $CaCl_2$. The cell wall was analyzed for cellulose content by the method of Updegraff (1969) and for uronic acid content by the method of Ahmed and Labavitch (1977). The cell wall was hydrolyzed in trifluoroacetic acid, essentially according to Jones Albersheim (1972), and the hydrolysate was deionized and subsequently analyzed for the total neutral sugar content by the method of Dygert et al. (1965). The individual sugars in the hydrolysate were analyzed by derivatization to the aldonitrile acetates and analysis by gas chromatography–mass spectrometry (Li et al., 1977).

**Isolation of a Cell Wall Fermenting Organism of Human Origin.** The bacterial growth medium which was employed in the isolation process was developed by Varel and Bryant (1974) and adapted by Salyers et al. (1977b). Agar was deleted from the media except as indicated. In this defined medium the peanut cell wall was substituted for glucose at 0.5% and served as the sole carbohydrate source. The medium was prepared anaerobically under $N_2$ and the cell wall was autoclaved in the medium prior to inoculation.

An inoculum of ~0.5 g wet weight of human feces was introduced into the bacterial growth medium, and the inoculated medium was incubated at 37 °C under a $N_2$ atmosphere and monitored for evidence of cell wall degradation. $N_2$ was employed as the headspace gas to avoid the pH effects encountered with $CO_2$ use. The turbidity and the pH of the medium and the physical appearance of the cell wall were monitored for evidence of cell wall utilization. This mixed fecal inoculum was carried through a transfer scheme (described more fully under Results) to isolate individual organisms capable of degrading the peanut cell wall.

**Preparation of Microbial Enzyme Fractions.** A bacterium, capable of degrading the cell wall, was isolated via the above procedure. Extracellular, intracellular, and pellet fractions of this organism were prepared for subsequent enzyme assay. The procedure involved is outlined in Figure 1. An actively growing culture of the organism was inoculated at 5% into the cell wall medium (or a substitute carbohydrate as indicated in the text). The inoculated medium was incubated under a $CO_2$ atmosphere at 37 °C for 14 h. The culture was centrifuged at 17500*g* and the supernatant fluid saved for testing as the extracellular enzyme fraction. The pelleted cells and plant cell wall were washed in 0.05 M phosphate buffer (pH 6.8) and resuspended in buffer containing 1 mM dithiothreitol. This cell suspension was placed in an ice bath and sonicated in four 1-min pulses interspersed with 1-min pauses. The disrupted cells were centrifuged and the supernatant fluid was retained for assay as the intracellular enzyme fraction. The pelleted material, composed of bacterial debris and residual peanut cell wall, was washed twice in buffer and resuspended for assay.

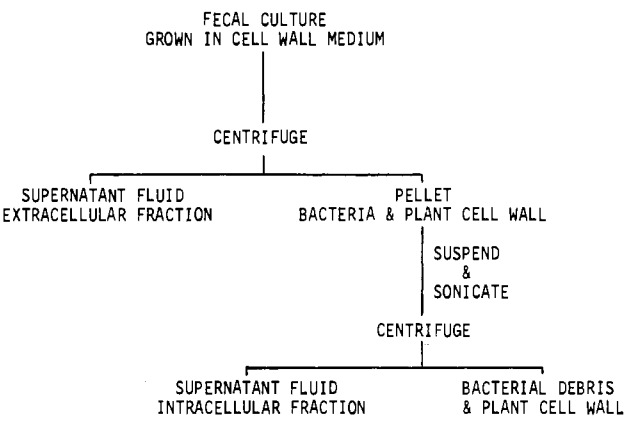


**Figure 1.** Scheme for the preparation of microbial enzyme fractions.

**Assay of the Crude Microbial Enzyme Fractions.** The polysaccharides employed as substrates included cellulose BW-40 (Brown Co., Berlin, NH), arabinogalactan (St. Regis Co., Libby, MN), psyllium hydrocolloid (Searle Laboratories, Chicago, IL), xylan (Sigma, St. Louis, MO), polygalacturonic acid (Sunkist Growers, Inc., Ontario, CA), and the cell wall. The substrate polysaccharide was added to 0.05 M sodium phosphate buffer at pH 7.0 to a final concentration of 10 mg/mL, autoclaved, and thoroughly mixed. One volume of the extracellular, intracellular, or pellet enzyme fraction was added to 1 volume of the substrate solution. This enzyme–substrate mixture was incubated at 37 °C for 18 h and then boiled for 10 min to terminate the reaction. Controls for each variable were identically prepared except that they were boiled prior to the incubation. Following incubation, the samples were centrifuged, and the supernatant fluid was tested in triplicate by the method of Dygert et al. (1965) to determine the total sugar liberated from the cell wall. The liberated sugar was reported as glucose equivalents in micrograms per milliliter. The supernatant fluid of selected enzyme–substrate incubations was examined by thin-layer chromatography (TLC) and gas chromatography (GC) to determine the individual sugars which had been released. The TLC employed separation on a phosphate buffered plate using a solvent system of 1-butanol–acetone–water (4:5:1) (Ghebregzabher et al., 1976). The GC employed the method of Li et al. (1977) as previously described.

## RESULTS

**Peanut Cell Wall Composition.** The yield of indigestible peanut residue by a modification of the method of Hellendoorn et al. (1975) was 5% of the roasted peanut

Table I. Composition of the Peanut Cell Wall

| component | % by wt |
|---|---|
| moisture | 7.4 ± 0.2[a] |
| cellulose | 20.8 ± 1.6 |
| uronic acid | 20.5 ± 0.6 |
| noncellulosic neutral sugars | 30.8 ± 2.5 |
| residue[b] | 11.5 |

[a] Values represent the mean and the standard deviation ($n = 3$). [b] Residue from the trifluoroacetic acid hydrolysis of the cell wall, corrected for cellulose content. This probably consists of protein, ash, and Maillard browning products.

Table II. Distribution of the Noncellulosic Neutral Sugars of the Peanut Cell Wall As Determined by Gas Chromatography-Mass Spectrometry

| sugars | % of noncellulosic neutral sugars |
|---|---|
| mannose | 2.6 ± 0.3[a] |
| fucose | 5.5 ± 3.5 |
| rhamnose | 7.2 ± 0.5 |
| galactose | 8.2 ± 1.6 |
| glucose | 11.0 ± 0.8 |
| xylose | 20.4 ± 1.2 |
| arabinose | 44.8 ± 3.7 |

[a] Values represent the mean and the average deviation ($n = 2$).

weight, while Hellendoorn had a yield of 7.7%. This indigestible peanut residue will subsequently be referred to as the cell wall.

The cell wall composition is given in Table I. About 9% of the cell wall material was not accounted for, presumably representing the combined errors of the methods employed. For example, the noncellulosic neutral sugar content of 30% is a composite of several sugars but is expressed as equivalents of glucose. Table II indicates the distribution of these noncellulosic neutral sugars as determined by gas chromatography of a derivatized cell wall hydrolysate. The major sugars were arabinose and xylose in a ratio of 2:1.

**Isolation of a Cell Wall Fermenting Organism.** An inoculum of human feces, which was introduced into the anaerobic cell wall medium, caused a decrease in pH and a visible change in the appearance of the cell wall within 24 h. The cell wall became more compact in appearance and settled to the bottom of the tube. From this initial culture, eight serial transfers (0.5 mL of inoculum into 10 mL of fresh media) were made at 24-h intervals, in each case after noting a pH drop and an altered physical appearance of the cell wall. Inoculum from the eighth transfer was streaked onto plated medium (the minimal medium plus 1.5% agar and 0.5% cell wall) and incubated anaerobically. Eight organisms of different colony morphology, each capable of fermenting the cell wall, were subsequently isolated.

The organism which appeared to most rapidly degrade the cell wall, as judged by physical appearance, was selected for further study. It was submitted to the Virginia Polytechnic Institute Anaerobe Laboratory for identification. The organism, a Gram-negative rod, was identified as a member of the genus *Bacteroides*. Its test characteristics, as described in the Anaerobe Laboratory Manual (Holdeman et al., 1977), did not correspond to any established *Bacteroides* species.

**Distribution of Enzyme Activity.** Enzyme fractions, prepared from the fecal isolate (Figure 1), were tested for their ability to liberate sugars from the cell wall substrate. Each of these fractions—extracellular, intracellular, and adsorbed enzymes—was able to solubilize cell wall sugars. Throughout six separate assays, the intracellular fraction showed the highest level of activity, with 62 ± 12% of the total activity. The pellet fraction had 27 ± 8% and the extracellular fraction had the lowest activity at 11 ± 5%. Because the intracellular enzyme fraction demonstrated the highest level of activity on the cell wall, it was the focus of the remaining assays. On an average, during an 18-h incubation, 550 ± 106 μg/mL ($n = 10$) glucose reducing equivalents were released from the cell wall by intracellular enzymes. With the substrate present at 5 mg/mL, this indicates that ~11% of the cell wall was being degraded.

Table III. Intracellular Enzyme Activity of the Isolate Grown in the Cell Wall Medium

| polysaccharide substrate | reducing ends as glucose, μg $mL^{-1}$ $(18\ h)^{-1}$ | hydrolysis, % | no. of assays |
|---|---|---|---|
| cellulose (BW-40) | 26 ± 6[a] | 0.52[b] | 2 |
| arabinogalactan | 55 ± 20 | 1.1 | 2 |
| psyllium hydrocolloid | 10 | 0.20 | 1 |
| xylan | 191 ± 24 | 3.8 | 4 |
| polygalacturonic acid | 737 ± 154 | 14.7 | 2 |
| cell wall | 506 ± 88 | 10.1 | 3 |

[a] Values represent the mean and the standard deviation. Substrate concentration in all cases was 5 mg/mL. [b] Approximations assuming that complete hydrolysis of the substrates will yield 5000 μg/mL product.

Table IV. Sugars Liberated from the Cell Wall Substrate by Intracellular Enzymes from the Isolate Grown in the Cell Wall Medium

| sugar | TLC[a] | GC[b] |
|---|---|---|
| arabinose | –[c] | + |
| fucose | – | trace |
| galactose | – | + |
| uronic acid | ++ | ND[d] |
| glucose | + | + |
| rhamnose | – | + |
| xylose | ++ | ++ |

[a] Thin-layer chromatography in 1-butanol–acetone–water (4:5:1). [b] Separated as aldononitrile acetate derivatives. [c] (–) Not detected; (+) minor component; (++) major component. [d] The presence of the sugar was not detected because the methodology employed was not applicable to that particular sugar.

The intracellular enzyme fraction was also assayed for its ability to degrade selected polysaccharides with a structural resemblance to plant cell wall components. As indicated in Table III, under the conditions of this assay, minimal enzyme activity was present on cellulose, arabinogalactan, and psyllium hydrocolloid substrates.

The activity detected on xylan and polygalacturonic acid suggests that xylanase and polygalacturonase enzymes or enzyme systems are present. The activity of the intracellular enzyme fraction on the cell wall was further examined to determine which sugars were being liberated. Table IV indicates the individual sugars that the enzymes released from the cell wall. Because the amount of material available from enzyme assays was limited, quantitation of the individual sugars was not attempted. However, by observation of the color intensity of TLC spots and/or size of GC peaks, xylose and a uronic acid were repeatedly confirmed as the predominant degradation products.

**Examination of the Constitutive or Inducible Nature of the Intracellular Enzyme Activity.** In previous studies Salyers et al. (1977a) discovered that many strains of colon bacteria were able to ferment polysaccharide substrates through the induction of the appropriate enzymes, based on the fact that no activity was detected in bacteria grown on glucose or xylose. The fecal isolate was

Table V. Activity on the Cell Wall of Enzymes from the Isolate Grown in Various Media

| carbohydrate in medium | reducing ends as glucose, μg $mL^{-1}$ $(18\ h)^{-1}$ | hydrolysis, % |
|---|---|---|
| xylose | 97 ± 34[a] | 1.9[b] |
| glucose | 79 ± 31 | 1.6 |
| cell wall | 511 ± 69 | 10.2 |

[a] Values represent the mean and deviation from the mean ($n = 2$). [b] Approximations as in Table III.

Table VI. Activity on the Cell Wall of Enzymes from the Isolate Grown on the Cell Wall for Varying Time Periods

| time, h | reducing ends, μg $mL^{-1}$ $(18\ h)^{-1}$ |
|---|---|
| 4 | 540 ± 37 |
| 11 | 615 ± 06 |
| 14 | 607 ± 67 |

grown in media containing the readily fermentable sugars glucose or xylose, as well as in a medium containing the complex cell wall substrate, to determine if the activity of the intracellular enzyme fraction was inducible. The intracellular enzyme fraction of the organism which was grown on xylose or glucose liberated a small amount of sugar from the cell wall (Table V). These activities were interpreted as a measure of the ability of the constitutive enzymes to degrade the cell wall. The polysaccharidase enzymes produced by the organism grown on the cell wall demonstrated a marked increase in cell wall degrading activity over that of the constitutive enzymes (Table V), suggestive of enzyme induction.

For determination of how rapidly these cell wall degrading enzymes appeared, the organism was grown on the cell wall for times ranging from 4 to 14 h, and the intracellular enzymes were assayed for cell wall degrading activity. The activities on the cell wall of intracellular enzymes from organisms grown on glucose (Table V) were regarded as base-line values. As shown in Table VI, within 4 h of contact with the cell wall, the organism produced enzymes capable of degrading the cell wall. Additional time of contact of the organism with the cell wall produced only a small additional increase in enzymatic cell wall degrading activity. The time course of enzyme production was not studied further.

## CONCLUSIONS

The evidence which is presently available on the cell wall structure of several plant species indicates that the structure and composition of the cell wall is unique to each plant source. The intact plant cell wall is an integrated structure of cellulose, hemicellulose, and pectins which also contains some protein, lignin, and other noncarbohydrate components. The cell wall is not digestible by human digestive enzymes and in most foods provides the bulk of the food residue which enters the colon. For utilization of the cell wall as a carbon source, the saccharolytic bacteria which inhabit the colon must have the capability to attack a wide variety of glycosidic linkages.

Salyers et al. (1977a) had previously noted that organisms capable of enzyme induction were at an advantage in utilizing the constantly changing mixture of polysaccharide substrates which passed through the colon. In the present study, the *Bacteroides* organism studied contained constitutive polysaccharidases with a limited ability to attack the peanut cell wall. Growth of the organism on the cell wall for as short as 4 h resulted in the apparent induction of xylanase and polygalacturonase. The sharp increase in enzyme activity in response to a new substrate, as observed here, is characteristic of enzyme induction. Salyers et al. (1977a) have reported that the polysaccharidases induced on model polysaccharide substrates tended to be intracellular. The enzyme activities measured in the present study were also predominantly intracellular, but there was significant activity in both the extracellular and pellet fractions.

Regarding the sequence of enzyme on the cell wall, Talmadge et al. (1973) noted that degradation of isolated sycamore cell wall could only be initiated by an endopolygalacturonase. This enzyme released ~16% of the cell wall as soluble products and disrupted the pectic framework of the cell wall, thus exposing further sites for enzyme attack. In the present study, the enzyme activities which were present after incubation of the organism with the cell wall for as short as 4 h included both polygalacturonase and xylanase. The extent of the degradation of the cell wall by the intracellular enzyme, during the course of an 18-h incubation, was ~11% of the cell wall substrate. More importantly, perhaps, the extent of the utilization of the cell wall by the intact organism was sufficient to cause a visible change in the cell wall within 14 h. Such an evident change in the cell wall appearance is suggestive of changes in the physical properties of the cell wall as well.

This investigation provides evidence that a bacterium isolated from human feces and belonging to the genus *Bacteroides* is capable of degrading an intact and insoluble plant cell wall. Although *Bacteroides* species predominate in the human colon, the particular organism utilized in the present study was not identified as one of the *Bacteroides* species commonly found in the colon. Hence, the ecological significance of the organism remains unknown. Nevertheless, the results do serve as a model for anaerobic degradation of plant cell walls or similar "dietary fiber" components.

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Reviewers have suggested various alternative research schemes and experiments which they feel may more effectively establish the conclusions drawn. As it seems impractical at this time to suggest extensive additional experimentation to answer the questions raised by the reviewers, we are publishing the papers essentially as delivered [Editor]. Other papers presented at the symposium were "Dietary Fiber in Cereals: Chemical, Enzymatic, and in Vivo Observations" (R. M. Saunders, E. Hautala, and E. A. Elliston), "Cereal Brans as Dietary Fiber Sources and Their Physiological Effects in Humans" (G. E. Inglett), "Changes in Dietary Fiber of Cereals during Processing" (S. K. Skarsauna and D. R. Schaller), "A Systems View of the Structure and Function of Dietary Fiber" (Ingemar Falkehag), and "The Physico-chemical Properties of Lignins and Their Potential Roles in Dietary Fiber" (B. A. Pethica and P. Zuman).

# ARTICLES

## Synthesis and Assessment of Three Compounds Suspected as Egg Aroma Volatiles

Victor Gil and Alexander J. MacLeod*

Previous work provided evidence of the occurrence of three novel compounds in the aroma volatiles of the hen's egg, namely, 1,2,4-trithiolane, 2-butylindan, and *O*-decylhydroxylamine. Here the synthesis of these three compounds is reported, but only the first two were confirmed as genuine components of egg volatiles. This is the first report of 1,2,4-trithiolane as a food volatile.

During our analysis of the volatile flavor components of the hen's egg, 65 compounds were positively identified (MacLeod and Cave, 1975), but in addition two novel and interesting components were only very tentatively assigned and could not therefore be reported, namely, 1,2,4-trithiolane and *O*-decylhydroxylamine. Stronger evidence was obtained for another component, 2-butylindan, but since this compound was somewhat unexpected, confirmation was desirable. All identifications were originally accomplished by GC–MS and included initially computer matching of spectra with a library file. The matching process was based on the 10 most intense peaks in the spectrum but was limited within a specified molecular weight range determined by inspection of the unknown spectrum. In addition, the selection of reference spectra was further limited to those in which the base peak of the unknown spectrum was also present as one of the six most intense peaks of the library spectrum. Although *O*-decylhydroxylamine showed an excellent match on this basis (0.85, with 1.0 representing perfect agreement between unknown and reference spectra), since the system was relatively unsophisticated this high score was mainly due to the alkyl fragments which dominate the spectra of many compounds possessing long-chain hydrocarbon substituents. Hence, the only certain deduction from the computer matching was that this egg component contained a labile long-chain alkyl group (probably decyl) and there was little real evidence for the particular class of compound suggested. This emphasizes the deficiency of these types of computer matching systems for mass spectra. However, more detailed examination of the full spectrum in question showed that the assignment from the computer matching was not entirely unreasonable and was certainly worthy of further consideration. Clearly, though, such a compound would be very unexpected as an aroma volatile.

With regard to the alkylindan, the computer matching was again very high (0.84), but for the decyl derivative. This seemed particularly unreasonable on the basis of its expected low volatility, and on careful inspection of the sample spectrum it seemed more likely that the unknown component might be a butylindan. It transpired that 2-decylindan was the only 2-alkylindan in the library file.

Although 3,5-dimethyl-1,2,4-trithiolane is well documented as a volatile of a number of foods, e.g., beef (Brinkman et al., 1972), potatoes (Buttery et al., 1970), and roasted filbert nuts (Kinlin et al., 1972), the computer match for the parent compound, previously undetected in

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