

Intestinal microbiota's naturally occurring enzymes and their effects on plant polysaccharide digestion

Poster Presentation at the Scripps Center for Integrative Medicine's 6th Annual Natural Supplements Conference, San Diego, California. January 22-25, 2009.

Christy L. Duncan, Gabriela Luta, Jane Ramberg, Tony X. Zhang, Robert A. Sinnott

INTRODUCTION

Human gastrointestinal (GI) bacteria, which provide extensive benefits for their host, largely rely on plant polysaccharides (PS)—indigestible by human enzymes—as their energy source. Because certain PS function as biological response modifiers, supporting host immune function, there is intense interest in better understanding their disposition in the GI tract. We reported that human fecal bacteria can partially hydrolyze a mixed polysaccharide dietary supplement (MSS) and two of its constituents, aloe vera gel powder (AVP) and larch arabinogalactan (LAG) *in vitro*; *Enterococcus faecium* best competed for these substrates.¹ Using a model that simulates the human GI tract (SHIME), we recently found that the MSS and a similar MSS containing an aloe vera gel extract (AVE) exerted prebiotic effects, supporting the growth of *Lactobacillus* and *Bifidobacteria* species.² To better understand the breakdown of PS in the gut, this *in vitro* study examined the digestion of both MSS product constituents [LAG, AVP, AVE, gum ghatti (GGH), gum tragacanth (GTR), fucoidans (FCS)] by GI tract bacterial glycosylhydrolases³ [α -1,6-mannosidase (α -1,6-M), α -1,2,3-mannosidase (α -1,2,3-M), α -1,2-fucosidase

(α -1,2-F), β -1,3-galactosidase (β -1,3-G), β -glucosidase and β -mannosidase].

METHODS

α -1,6-M, α -1,2,3-M, α -1,2-F and β -1,3-G were purchased from New England BioLabs, Inc. β -glucosidase and β -mannosidase were cloned by our research team. 50mM citrate buffer plus 0.01% Tween20 (pH adjusted according to enzyme used) was used to dilute each enzyme to achieve an activity of 4 units; preparation is summarized in Table I. Duplicate samples of weighed PS were suspended in buffer (LAG, AVP, and FCS ~ 10 mg/ml; AVE, GGH and GTR ~ 2 mg/ml), shaken vigorously and placed in a 37°C water bath for 1h. Enzyme solutions were then added to each PS solution and mixed with a pipette. For controls, an equivalent amount of deionized water replaced the enzyme solution for each PS. Experimental and control mixtures were placed into the 37°C water bath for 2h. Five mL aliquots were removed from each mixture, transferred to test tubes, heated at 100°C for 15 min and centrifuged for 15 min at 4,000 rpm. Supernatants were filtered through 0.2 μ m nylon syringe filters and MW distributions were assessed using high pressure liquid chromatography.

Table I Enzyme preparation

Enzyme	Enzyme: cloned from; expressed by	Amount of stock used for dilution (μ L)	Amount of buffer (μ L)	Buffer pH	Amount of enzyme added to PS (μ L)
α -1,6-mannosidase (α -1,6-M)	<i>Xanthomonas manihotica</i> ; <i>E.coli</i>	20	980	4.58	100
α -1,2,3-mannosidase (α -1,2,3-M)	<i>X. manihotica</i> ; <i>E.coli</i>	10	150	5.54	4
α -1,2-fucosidase (α -1,2-F)	<i>X. manihotica</i> ; not provided by manufacturer	2	38	5.56	4
β -1,3-galactosidase (β -1,3-G)	<i>X. manihotica</i> ; <i>E.coli</i>	5	45	4.55	4
β -glucosidase	<i>Bacteroides fragilis</i>	No dilution of enzyme	No dilution of enzyme	6.53	32
β -mannosidase	<i>B. fragilis</i>	No dilution of enzyme	No dilution of enzyme	5.54	53.3

RESULTS

The results are provided in Table II. A representative sample of chromatograms is provided in Figures 1-18.

Table II Digestion of PS

	α -1,2-F		α -1,6-M		α -1,2,3-M		β -1,3-M		β -glucosidase		β -mannosidase	
	Digestion	Generation of smaller saccharides	Digestion	Generation of smaller saccharides	Digestion	Generation of smaller saccharides	Digestion	Generation of smaller saccharides	Digestion	Generation of smaller saccharides	Digestion	Generation of smaller saccharides
LAG	++	++	++	++	++	++	++	+	++	++	++	++
AVP	+	+	+	+	+	+	+	+	+	++	++	++
GGH	+	+	++	++	+	++	+	+	++	++	++	++
GTR	++	++	++	++	+	+	++	+	+	++	++	++
FCS	++	+	+	-	++	+	+	+	+	++	+	++
AVE	NA	NA	+	++	+	+	-	+	++	++	++	++

+ = minimal activity
 ++ = substantial activity
 NA = data not available
 - = no activity

Figures 1-18

Figure 1 LAG
 α -1,2-F digestion

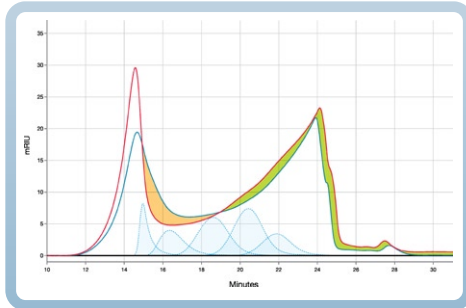


Figure 2 LAG
 α -1,6-M digestion

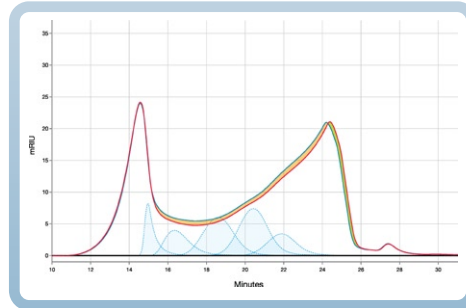


Figure 3 LAG
 α -1,2,3-M digestion

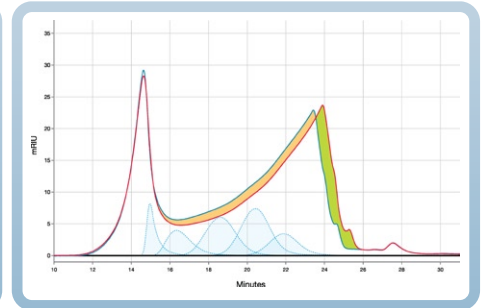


Figure 4 LAG
 β -1,3-G digestion

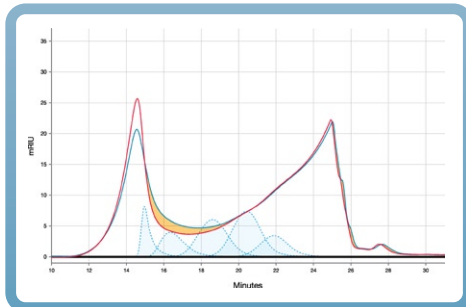


Figure 5 LAG
 β -glucosidase digestion

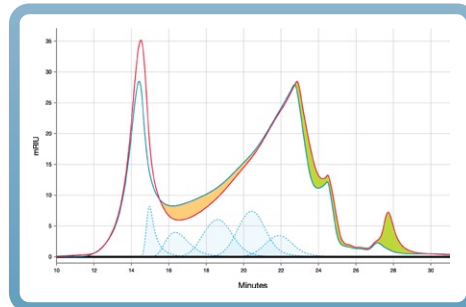


Figure 6 LAG
 β -mannosidase digestion

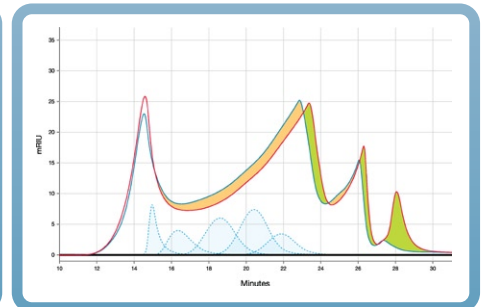


Figure 7 AVP
 β -mannosidase digestion

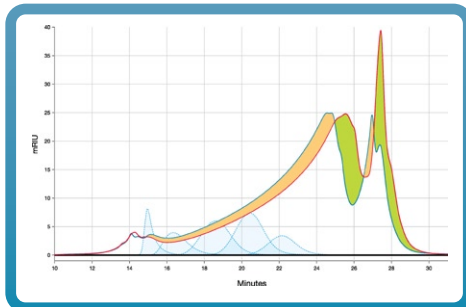


Figure 8 GGH
 α -1,6-M digestion

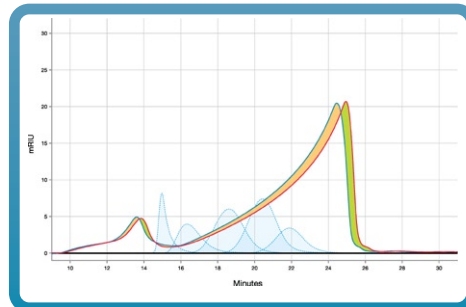
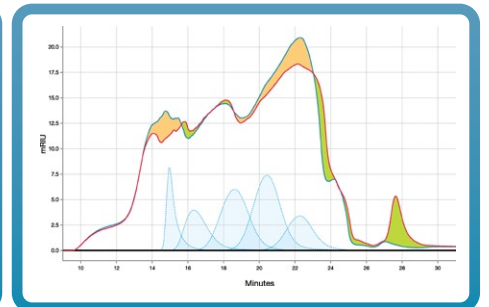


Figure 9 GGH
 β -glucosidase digestion



■ = control ■ = experimental ■ = digestion ■ = generation of lower MW PS

Figures 1-18 cont.

Figure 10 GGH
β-mannosidase digestion

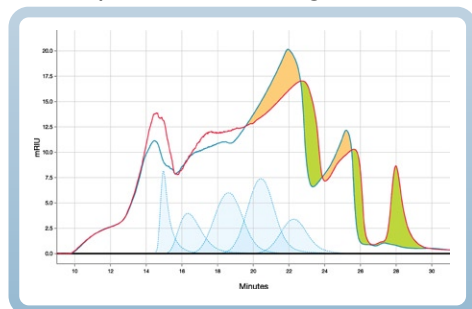


Figure 11 GTR
α-1,2-F digestion

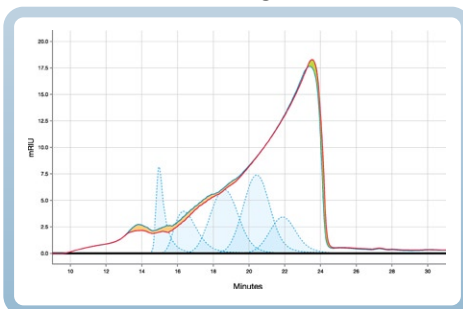


Figure 12 GTR
α-1,6-M digestion

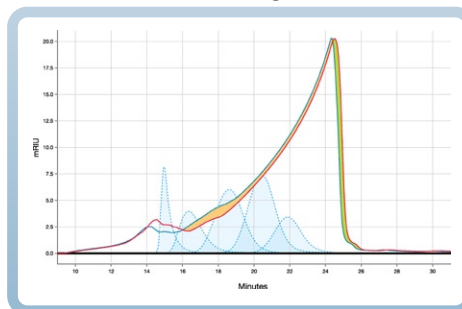


Figure 13 GTR
β-glucosidase digestion

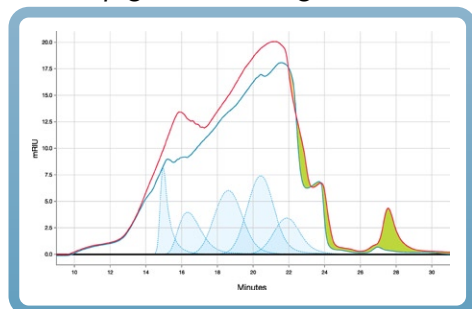


Figure 14 GTR
β-mannosidase digestion

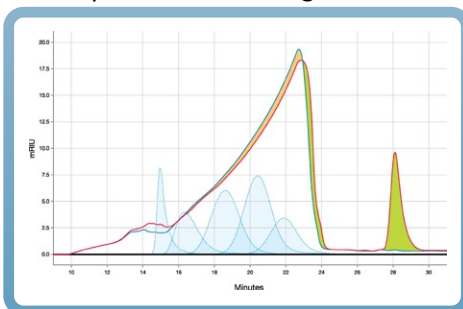


Figure 15 FCS
β-glucosidase digestion

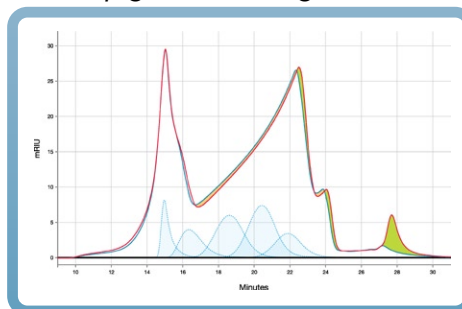


Figure 16 FCS
β-mannosidase digestion

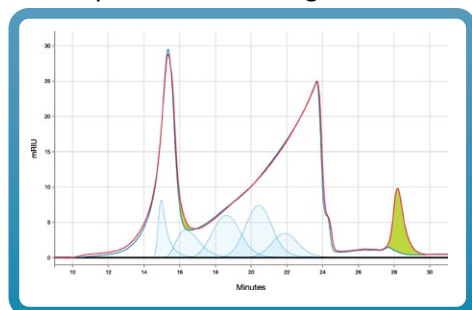


Figure 17 AVE
β-glucosidase digestion

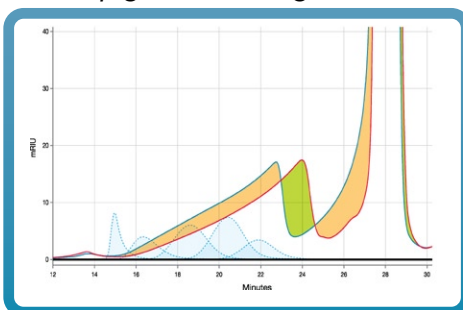
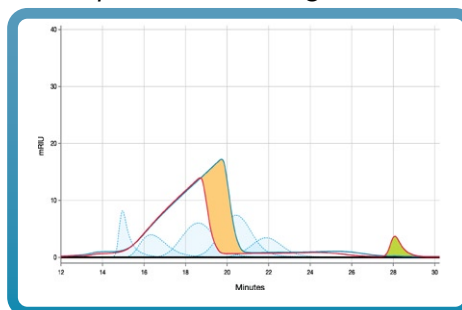


Figure 18 AVE
β-mannosidase digestion



■ = control ■ = experimental ■ = digestion ■ = generation of lower MW PS

DISCUSSION

Previous studies indicate that dietary supplements composed of the PS investigated in this study are utilized by human colonic bacteria.^{1,2} This is the first study to investigate the digestion of these PS individually by enzymes secreted by human GI bacteria.

LAG is reported to be composed of a β-D-(1,3)-galactan backbone with galactose and arabinose side chains.⁴ LAG was partially digested by all six enzymes (Figures 1-6). Digestion by β-1,3-G was expected; digestion by the remaining five enzymes suggests the presence of additional monosaccharides.

Previous authors have reported that aloe vera gel is rich in mannose, but also contains significant amounts of glucose

and small amounts of galactose, xylose, arabinose (or fucose) and rhamnose. β-linked mannan polysaccharides predominate.^{5,6,7} We recently reported that glucuronic and galacturonic acids are also present in fresh freeze-dried gel;⁸ and we reported that the sugar composition and MW of aloe vera gel products are significantly affected by processing methods.⁸

Production of AVP powder retains all aloe monosaccharides, with the exception of glucuronic acid and galactose.⁸ Predominant digestion of AVP by β-mannosidase was expected for a product expected to be rich in β-linked mannans (Figure 7).

GGH is composed primarily of arabinose, galactose, mannose, xylose and glucuronic acid, with mainly

α -D-mannopyranose and 1,6-linked β -D-galactopyranose units.⁹ α -1,6-M, β -glucosidase and β -mannosidase digested GGH (Figures 8-10); minimal digestion was apparent for the remaining three enzymes. Digestion by α -1,2-F (not represented) and β -glucosidase (Figure 9) was not expected, since glucose and fucose have not been reported in this gum.

GTR, composed of galactose, galacturonic acid, arabinose, xylose and fucose^{10,11} was partially digested by all enzymes. α -1,2-F (Figure 11) and β -1,3-G (not represented) digestion was expected because GTR contains fucose, galactose and galacturonic acid (oxidized form of D-galactose). Digestion with mannosidases and glucosidase (Figures 12-14) are surprising, but possibly explained by the LAG + galactose backbone that makes up tragacanthin in GTR. The same enzymes which utilized LAG above should utilize the LAG in tragacanth.

FCS is 55% carbohydrate (fucose, xylose, galactose, glucose, mannose and uronic acids) and 41.5% sulphate,^{11,12,13} with an α -linked skeleton.¹⁴ We found digestion by all enzymes, which agrees with the reported monosaccharide composition of FCS. Indication of β -linkages are also present (Figures 15-16).

AVE, predominantly mannose (β -linked mannans) with small amounts of glucuronic acid, xylose, galacturonic acid, galactose, glucose, glucosamine, fucose, rhamnose and arabinose⁸ was partially digested by all enzymes, which was expected (Figures 17-18).

Generation of oligosaccharides and monosaccharides are represented in Table II and Figures 1-18. This data further supports the break down of these large PS into smaller saccharides.

These results demonstrate the abilities of known GI tract bacterial glycosylhydrolases to digest PS, and provide additional structure and composition information for these PS. The generation of monosaccharides by α -glucosidase and β -mannosidase for most PS suggests that these two enzymes have the greatest ability to digest these PS.

ACKNOWLEDGEMENT

The authors would like to thank Jennifer Aponte for her assistance with the preparation of this poster.

REFERENCE LIST

1. Sinnott RA, Ramberg J, Kirchner JM, et al. Utilization of arabinogalactan, aloe vera gel polysaccharides, and a mixed saccharide dietary supplement by human colonic bacteria *in vitro*. *Int J Probiotics Prebiotics* 2007; 2(2/3): 97-104.
2. Verstraete W. *In vitro* evaluation of the prebiotic effect of two mixed polysaccharide dietary supplements using the TWINSHIME setup. Report 11.26.08.
3. Xu J, Gordon JI. Honor thy symbionts. *Proc Nat Acad Sci USA* 2003; 100(18): 10452-9.
4. Whistler, R.L, Richard EL, Hemicelluloses. The Carbohydrates. 2nd ed. Pigman W, Horton D, Eds.; Academic Press; New York, 1970; 447-469.
5. Talmadge J, Chavez J, Jacobs L, et al. Fractionation of Aloe vera L. inner gel, purification and molecular profiling of activity. *Int J Immunopharmacol* 2004; 4(14): 1757-73.
6. Waller GR, Mangiafico S, Ritchey CR. A chemical investigation of Aloe barbadensis miller. *Proc Okla Acad Sci* 1978; 58:69-76.
7. Femenia A, Sanchez ES, Simal S, et al. Compositional features of polysaccharides from Aloe vera (*Aloe barbadensis miller*) plant tissues. *Carbohydr Polymers* 1999;39(2): 109-17.
8. Duncan C, Ramberg J, Sinnott R. Striking differences in Aloe vera gel carbohydrate composition, molecular weight and particle size distributions following processing will not be addressed by dietary supplement GMPs. Scripps Center for Integrative Medicine, San Diego, California; Poster Presentation at the 5th Annual Natural Supplements Conference, January 17-20, 2008, 2008.
9. Glicksman M. Gum Ghatti (Indian gum). In Food Hydrocolloids. Glicksman M, Ed.; CRC Press: Boca Roton, 1983; 31-37.
10. Santos EM. Final report on the safety assessment of Tragacanth gum. *J Am Coll Toxicol* 1987; 6(1):22.
11. Bruneton J. Pharmacognosy, Medicinal Plants. 2nd ed. Intercept, Ltd: New York, NY, 1999.
12. Koo J-G. Structural characterization of purified fucoidan from *Laminara religiosa*, sporophylls of *Undaria pinnatifida*, *Hizikia fusiron* and *Sagassum fulvellum* in Korea. *J Korean Fish Soc* 1997; 30(1): 128-31.
13. Yang C, Chung D, You SG. Determination of physicochemical properties of sulphated fucans from sporophyll of *Undaria pinnatifida* using light scattering technique. *Food Chem* 2008; 111(2): 503-7.
14. Kim WJ, Kim SM, Lee YH, et al. Isolation and characterization of marine bacterial strain degrading fucoidan from Korean *Undaria pinnatifida* sporophylls. *J Microbiol Biotechnol* 2008; 18(4): 616-623.