

Human Colonic Bacteria Can Utilize Stabilized Aloe Vera Gel Polysaccharides and a Mixed Saccharide-Based Glyconutritional Dietary Supplement, Advanced Ambrotose™

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INTRODUCTION

Some plant-derived polysaccharides are among the best known and most potent immunomodulatory substances, impacting both humoral and cellular immune responses. Certain polysaccharides in aloe vera gel have demonstrated such effects. In addition, mixed saccharide-based glyconutrient supplements, formulated to support glycoconjugate synthesis, can exert a wide range of effects on human health, exceeding expectations derived from immunomodulatory polysaccharide (IP) literature.

The means by which IPs exert their effects is not well understood. The literature suggests, however, that many effects originate in the GI tract, home to 70% the immune system.¹ A growing body of literature continues to confirm that a healthy population of GI bacteria, which primarily relies on plant polysaccharides as a food source, is critically important for human health. To begin to understand the fate of oral IPs in the human GI tract, this *in vitro* study assessed colonic degradation of 1) stabilized high molecular weight aloe vera gel polysaccharides (AVP); and 2) a glyconutritional dietary supplement (Advanced Ambrotose™) (AA). Degradation of larch arabinogalactan (LAG) was assessed as a positive control. LAG and AVP are components of AA.

METHOD

Experimental Polysaccharide Sample Preparation

Weighed samples of experimental polysaccharides (EP): 1) stabilized high molecular weight aloe vera gel polysaccharides (AVP); 2) Advanced Ambrotose™ (AA)* or 3) Larch arabinogalactan (LAG) (positive control), were thoroughly mixed in deionized water before dialysis for 20 h at 4° C in Spectrum MWCO 6,000-8,000 tubing. Dialyzed samples were frozen for one hour prior to being freeze dried.

*a patented formula containing AVP, LAG, Anogeissus latifolia stem gum, Astragalus gummifer stem gum and Undaria pinnatifida extract.

Fecal Cultures

Preparation Fecal samples, provided by 4 healthy pre-screened[†] adults, were confirmed negative for the following pathogens: *Clostridium difficile*, Salmonella, Shigella, Campylobacter, Vibrio, Yersinia, and *E. coli* 0157. Ten gram samples were mixed with 750 ml Brain Heart

Infusion (BHI) and mixed in a Waring blender for four 30 second pulses before six hour incubation at 37° C. Aliquots were then either frozen in 20% glycerol or used to seed a continuous flow fermentor in a 3X3 day growth protocol (below). Frozen samples were thawed, diluted in 1XE salt media[‡] and pre-incubated in 1XE media with the EP for 48 hours before incubation in the 3X3 day growth protocol.

3X3 day growth protocol EPs were suspended by agitation in deionized water for 2 h at room temperature. The EP suspension was then diluted in 1XE media (1% w/v).[€] Under anaerobic conditions, 20 ul of fecal suspension was added to 2 ml of the 1% EP in 1XE media and left to incubate for 3 days under anaerobic conditions at 37° C. Three days later, 20 ul of the culture was passed to 2 ml of fresh 1% EP and passed again on day six. Nine days after the start of culture (three days after the final pass), bacteria were collected by centrifugation and snap-frozen in a dry ice ethanol. Supernatants were saved for analysis of EP composition by HPLC refraction detection. Controls were processed in an identical manner except that they were not inoculated with feces. Day nine bacteria were harvested and processed for 16srDNA identification.

[†]Subjects were eliminated if they answered “yes” to any of the following questions: 1) Are you older than 45? 2) Are more than 25% of your meals fast food? 3) Have you taken antibiotics within the last 12 months? 4) Do you have irregular bowel movements? 5) Have you been treated for GI problems in the last 5 years? 6) Do you use OTC medicines for constipation, stomach or bowel cramps, or diarrhea? 7) Have you had food poisoning in the last 6 months? 8) Do you think carefully about where you are going to eat because you are worried about getting sick?

[‡]1XE media: 2 g citric acid + 10 g K₂HPO₄ + 3.5 g NaNH₄HPO₄•4 H₂O + 0.02 g MgSO₄•7 + 1 liter deionized water

[€]To ensure that anaerobic conditions were maintained throughout, all manipulations were conducted in an anaerobic chamber flooded with nitrogen gas for 15 minutes before use and all media (liquids and plates) were off-gassed in a nitrogen-flooded chamber for 15 minutes before use.

Size Exclusion Analysis of MW Standards and Continuous Flow Fermentor Supernatants

Pullulan standards (5,000; 10,000; and 800,000 MW) were added to deionized water (about 1 mg/ml) and analyzed by HPLC[€] to confirm that EP dialysis had eliminated the

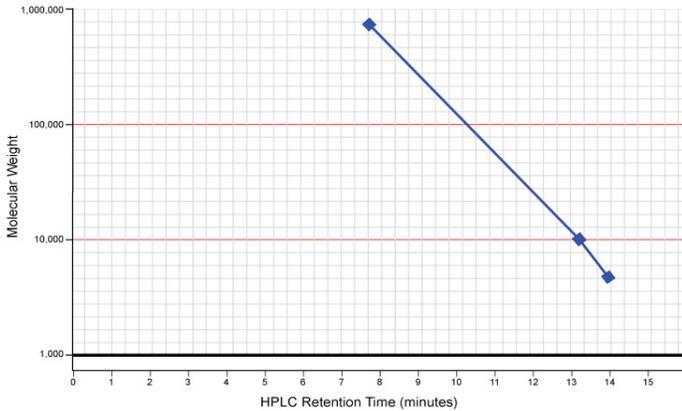
smaller saccharides (<6,000 MW) and to allow estimation of the MW of EPs and supernatants. Supernatants were thawed at room temperature, added to deionized water and filtered with 0.2µm polypropylene Whatman Mini-UniPrep™ filters before HPLC analysis.

†All HPLC analyses were conducted on a Shimadzu HPLC with a Phenomenex BioSep-SEC-S-4000 column.

RESULTS

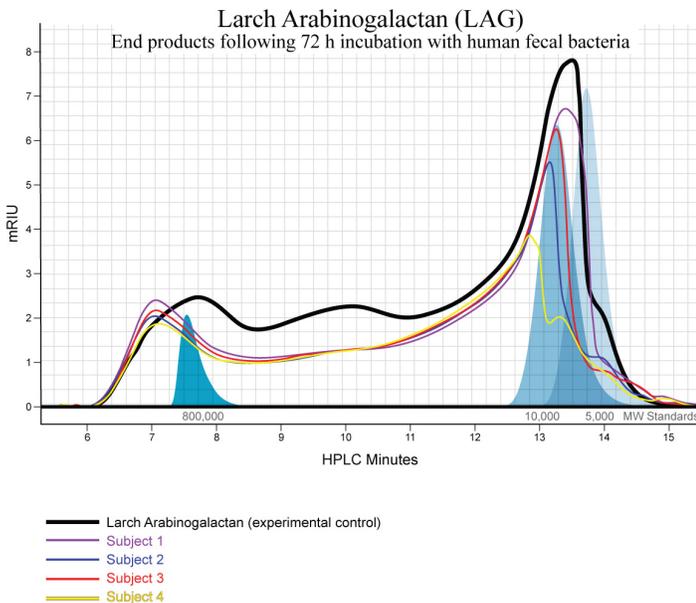
Figure 1 provides the HPLC retention time standard curve for Pullulan standards.

Figure 1



All four subjects' fecal cultures consumed LAG polymers equally well between the ranges of 800,000 to approximately 12,000 MW. Polymers less than 12,000 MW, however, were consumed in differing amounts (Figure 2).

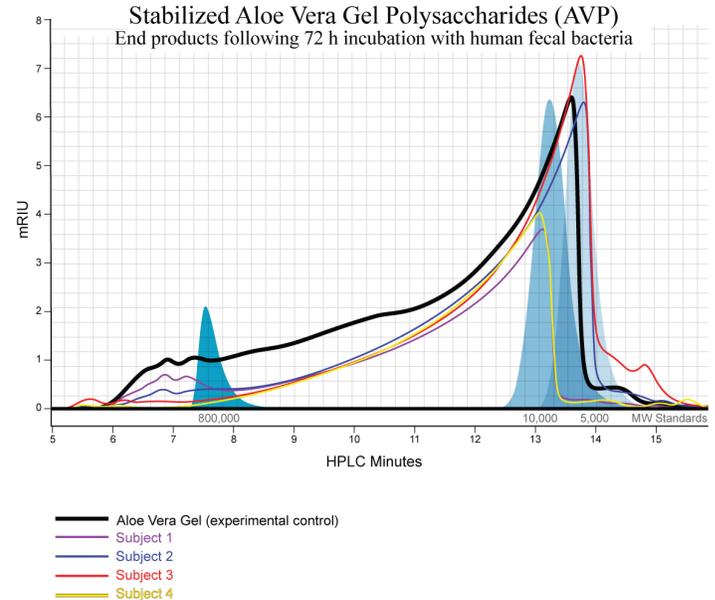
Figure 2



Polymeric components of AVP between approximately 1,000,000 and approximately 50,000 MW were almost

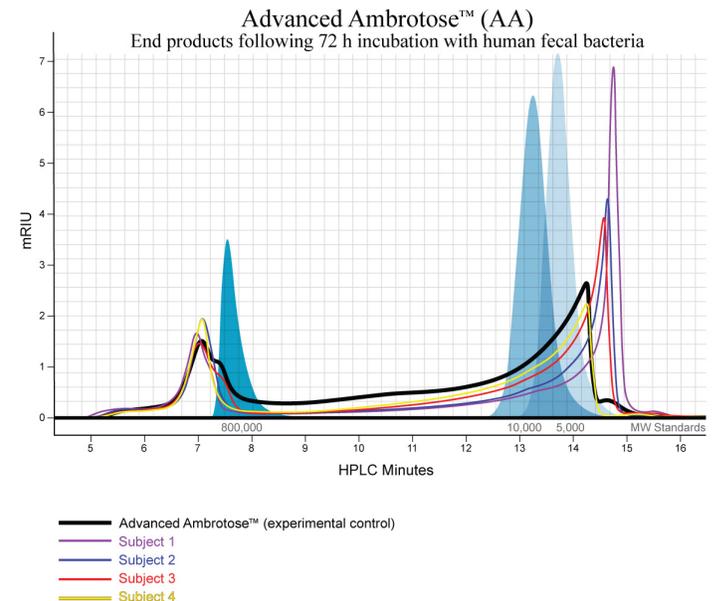
entirely consumed by fecal bacteria from all subjects, though there was some variability, particularly at the 1,000,000 MW range. Polymers <10,000 were consumed in a highly variable manner by different fecal cultures (Figure 3).

Figure 3



Polymeric components of AA of approximately 1,000,000 were not consumed, but polymers between 800,000 to 10,000 MW were consumed to varying extents by different cultures selected by AA. Interestingly, in three subjects, fecal culture degradation of AA, but not AVP or LAG, resulted in the accumulation of low molecular weight polymers ~1,000 MW that are not found in the control AA (Figure 4). This strongly suggests that AA evokes the

Figure 4



production of exported glycosidic enzymes that are able to reduce the size of polymers.

16srDNA bacteria identification data was incomplete at the time of this presentation and will be the subject of a subsequent publication.

DISCUSSION

The process of repeated passage of fecal bacteria into fresh medium that contains only EPs as an available carbon source selects for those bacteria that can utilize EPs for growth. Because bacteria can reproduce hundreds of times in nine days, those that can utilize EPs effectively and reproduce quickly will be selected over bacteria that may be able to utilize EPs but reproduce more slowly. Thus, the number of bacterial species present in culture after nine days may represent a relatively small number of species that are able to utilize EPs. Another important consideration is that an estimated 70% of bacteria that reside in the GI tract cannot be cultured under any conditions. It is thus likely that some species may be able to readily utilize EPs *in vivo* but cannot be identified using approaches such as the one used here. The DNA from recovered bacteria is now being sequenced to determine species that remain after a nine day exposure to only EPs as a carbon source.

The only approach that can fully determine the impact of a nutritional supplement on the gut biome is to evaluate the effects on the host. Clearly, a comprehensive chemical description of metabolic end-products produced by EP fermenting bacteria *in vitro* may provide the basis for understanding possible host effects. Chemical profiling of metabolic end-products produced by bacteria that can utilize EPs as a sole carbon source is now underway.

Previous studies have documented the utilization of arabinogalactan by human fecal bacteria.^{2,3,4,5} This study is consistent with the findings of the *in vitro* studies of Salyers et al.⁴ and Englyst et al.,⁶ who also noted incomplete degradation of arabinogalactan.

This is the first study to assess the utilization of AVP and AA by human fecal bacteria. An interesting finding is that there is great variation between individuals regarding the ability to utilize high molecular weight (> 1,000,000) components of AVP. Consumption of very large molecules with limited solubility suggests that bacteria with the ability to internalize these molecules are important. Internalization

suggests that some bacteria are able to apply multiple enzymatic pathways to unfold, degrade, and ultimately oxidize these highly complex and highly organized polysaccharides.

Another striking observation is that AA feeding leads to the abundant generation of relatively small (~1,000) polymers in the supernatant. Since the AA control is >5,000, generation of lower MW substances must arise from the degradation of larger molecules. This could occur either from degradation of large molecules by enzymes exported to the supernatant by bacteria or from secretion of small polymers by the bacteria themselves. What is quite surprising is that this phenomenon is not observed for either LAG or AVP, two major components of AA. This may suggest that the combination of these two EPs with the other ingredients in AA may lead to a unique phenotypic switch or to selection of a unique cohort of bacteria.

CONCLUSIONS

This *in vitro* study of four human subjects suggests that human fecal bacteria are capable of utilizing two immunomodulatory polysaccharide products (stabilized aloe vera polysaccharides and a mixed saccharide-based glyconutritional supplement, Advanced Ambrotose™). There was striking variability between individuals in their ability to utilize high MW (> 1,000,000 MW) aloe vera polysaccharides. Advanced Ambrotose™ feeding leads to the abundant generation of relatively small (~1,000) polymers. This phenomenon suggests that the combination of these two polysaccharides with the other ingredients in Advanced Ambrotose™ may lead to a unique phenotypic switch or to selection of a unique cohort of bacteria.

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