

## Abstract

Segmented filamentous bacteria (SFB) are host-specific commensal gut-microbes which are known to be potent inducers of the host adaptive immune system. By binding tightly to the host's small intestinal epithelial cells, at the time of weaning, SFB are known to induce the accumulation of anti-bacterial  $T_H17$  cells in the lamina propria, protecting the host from colonization by pathogenic and opportunistic microbes. Given that the small intestinal lumen is decorated with a rich coat of carbohydrates, it is possible that binding of SFB to the host may be mediated by protein-carbohydrate interactions. A bioinformatic analysis of predicted SFB adhesion proteins revealed P12260, a unique lipoprotein adhesin with a predicted carbohydrate binding domain. P12260 may facilitate SFB adhesion by binding to glycan residues found in intestinal mucins, extracellular matrix proteins, or on the epithelial cell surface. To better understand the function of P12260, two variants of this protein, His-tagged P12260M (derived from SFB in mono-colonized mice) and P12260T (derived from SFB in wild type Taconic mice), were recombinantly expressed and purified from *E. coli* using a combination of Nickel-Immobilized Metal Affinity chromatography (Ni-IMAC) and size exclusion chromatography (SEC) to greater than 99% purity. To probe potential ligands for P12260M and P12260T, the proteins were tested using two different procedures: insoluble polysaccharide pull-down with chitosan-monosaccharide conjugates and binding to cellulose-carbohydrate membranes. Binding studies with chitosan-monosaccharide conjugates revealed statistically significant trends for P12260M binding to L-fucose ( $p < 0.01$ ), L-rhamnose ( $p < 0.05$ ), and D-glucose ( $p < 0.01$ ). To overcome the need for excess reagents and low signal-to-noise ratios in the tested methods, a new carbohydrate microarray was synthesized using di-vinyl sulfone immobilization of monosaccharides to polyvinyl alcohol thin-films casted on glass slides. Finally, a *Bacillus subtilis* surface display system was developed to generate a high throughput method to screen the function of P12260 and other SFB surface proteins using mammalian cell culture systems *in vitro*.