Evaluating the Cellulolytic Properties of Novel Fibrobacteres isolates from Mammalian Herbivores

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Project Goals: Characterizing novel cellulose-degrading microbes is of great interest for the development of biofuels and bioproducts from cellulosic feedstocks. One group of bacteria includes the phylum Fibrobacteres, which are well known for the prolific cellulose-degrading properties. Surprisingly, our understanding of the entire phylum is limited to only two described species within the genus *Fibrobacter*. Here, we sought to expand our understanding of this important cellulolytic phylum by culturing novel isolates from a multitude of different herbivorous hosts. We tested each isolates ability to degrade cellulose and compared them to the type strain *Fibrobacter succinogenes*. This work expands on our existing knowledge about the Fibrobacteres and further provides access to an untapped diversity of novel cellulolytic enzymes.

Bacteria in the phylum Fibrobacteres are well known for the highly cellulolytic properties. For example, the type strain *Fibrobacter succinogenes* S85 is the most prolific cellulose degrader known. Non-culture based approaches have identified Fibrobacteres bacteria from the gastrointestinal tract of a wide range of mammalian herbivorous hosts, yet these species have thus far remained resistant to culturing efforts. Given the highly cellulolytic nature of the existing *Fibrobacter* species, we posited that species associated with mammalian herbivores would also exhibit similar properties. This idea is supported by the fact that *F. succinogenes* exists within the cow rumen, one of nature's most highly optimized plant biomass degrading ecosystems. To test this, we developed a novel culturing method and employed to obtain 45 new isolates from 11 different herbivorous hosts. Extensive phylogenetic analysis of these isolates expands our understanding of this phylum by revealing 6 new clades, with many representing new species and genera. Isolates tended to group by host, and between hosts there were distinct groupings into hindgut and foregut fermenters. We then tested each isolates ability to degrade both cellulose and hemicelluloses, and found that, like the type strain, all isolates were only able to degrade and utilize cellulose. These findings suggest that members of this phylum are cellulose specialists, as has been observed for the type strain. Further analysis of these isolate's fermentation products revealed that, like *F. succinogenes*, all cultures produced succinate as the major fermentation product, with lesser amounts of acetate and formate. Our results expand our understanding of the Fibrobacteres and provides insights into the ecology and evolution of a prolific cellulose-degrading specialist.

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