The Devil Lies in the Details: How Variations in Polysaccharide Fine-Structure Impact the Physiology and Evolution of Gut Microbes

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Abstract

The critical importance of gastrointestinal microbes to digestion of dietary fiber in humans and other mammals has been appreciated for decades. Symbiotic microorganisms expand mammalian digestive physiology by providing an armament of diverse polysaccharide-degrading enzymes, which are largely absent in mammalian genomes. By out-sourcing this aspect of digestive physiology to our gut microbes, we maximize our ability to adapt to different carbohydrate nutrients on timescales as short as several hours due to the ability of the gut microbial community to rapidly alter its physiology from meal to meal. Because of their ability to pick up new traits by lateral gene transfer, our gut microbes also enable adaption over time periods as long as centuries and millennia by adjusting their gene content to reflect cultural dietary trends. Despite a vast amount of sequence-based insight into the metabolic potential of gut microbes, the specific mechanisms by which symbiotic gut microorganisms recognize and attack complex carbohydrates remain largely undefined. Here, we review the recent literature on this topic and posit that numerous, subtle variations in polysaccharides diversify the spectrum of available nutrient niches, each of which may be best filled by a subset of microorganisms that possess the corresponding proteins to recognize and degrade different carbohydrates. Understanding these relationships at precise mechanistic levels will be essential to obtain a complete understanding of the forces shaping gut microbial ecology and genomic evolution, as well as devising strategies to intentionally manipulate the composition and physiology of the gut microbial community to improve health.

Introduction

Humans consume a broad range of polysaccharide-rich foods, not only in the form of plant material (cell walls and storage polymers) but also as animal connective tissue, food additives and even microbial and fungal products. Our intrinsic ability to digest the available repertoire of complex carbohydrate molecules remains limited to just starch, lactose and sucrose [1]. This metabolic decrement is due to a paucity of fiber-degrading enzymes encoded in the genomes of humans and other animals (for a recent overview, see Ref. [1]). Fortunately, we have co-evolved with a dense consortium of symbiotic distal gut microorganisms (microbiota), many of which have adapted to target these polysaccharides for their own nutrition. In return, we reap the benefits of these gut symbionts' largely fermentative metabolism, which produces short-chain fatty acids and other products that are absorbed in the colon as nutrients [2].

Of the dozens of different phyla of bacteria and archaea that exist on Earth, less than 10 are abundant in the guts of humans [3–5]. The Gram-positive Firmicutes are typically most numerous, followed by Gram-negative Bacteroidetes. Other common but less numerically abundant phyla include Actinobacteria, Verrucomicrobia and Proteobacteria, among others. The selection for a few taxonomic groups was probably ancient, since these same phyla are shared among other mammals and many invertebrates [4]. Moreover, at finer taxonomic levels (genus and species), the microorganisms found in human and animal guts are typically not present in environmental reservoirs, leading to the hypothesis that we have

co-evolved with many of these organisms and provide their only habitats [6].

The genomes of sequenced human gut bacteria and the metagenomes of the communities they compose reveal that our microbial symbionts have much more extensive armaments of polysaccharide-degrading enzymes than we do [1,5,7–9]. This is evident in both the numbers of enzymes present and the diversity of catalytic activities [1]. As a particularly striking example, the recently published 7.1-Mbp genome of *Bacteroides cellulosilyticus* WH2 contains a total of 424 glycoside hydrolases, polysaccharide lyases and carbohydrate esterases, which is ~25 times the number of human genome-encoded enzymes that are thought to be secreted into the gastrointestinal tract [10]. Of the 76 different CAZyme (*c*arbohydrate-*a*ctive en*zyme*) families (as defined in the Carbohydrate-Active Enzymes Database [11]) present in *B. cellulosilyticus* WH2, 56 are not represented in the human genome, highlighting the amount of metabolic expansion that even a single gut bacterium adds. Without this help from symbiotic bacteria, humans and other animals, ranging from termites to ruminants, would simply be incapable of assimilating nutrients from a substantial portion of dietary polysaccharides.

Despite a vast—and expanding—amount of sequence-based insight, precise mechanistic relationships between the enormous diversity of polysaccharides that enter our digestive system and the microbes that degrade them have been slower to develop. In this review, we consider several emerging facets of how symbiotic gut microorganisms assist humans and other animals with polysaccharide digestion. We focus first on the evolutionary benefit of this digestive symbiosis, subsequently outline the sensory and enzymatic mechanisms employed by various gut bacteria to distinguish these nutrients and conclude by discussing some recent data that imply the presence of finely adapted and niche-specific microbe–polysaccharide interactions in the gut, some of which are being driven by the lateral transfer of genes involved in polysaccharide degradation.

Old Questions Still in Need of Detailed Answers

The critical role of intestinal microorganisms in polysaccharide degradation became appreciated around the early 1940s when Robert Hungate, a pioneer in the field of anaerobic microbiology, explored the phenomenon of microbial cellulose degradation in the bovine rumen and termite gut [12]. With the advent of more facile anaerobic culturing techniques—including the development of the anaerobic chamber by Freter and colleagues in the 1960s [13]—pioneers in human gut microbiology, such as Freter, Holdeman and Moore, reported substantial viable recoveries (up to 46–93%) of the bacterial cells observable by direct microscopic counts [13,14]. As a testament to the experimental skill of these scientists, the lists of most abundant human gut bacterial species that they found in early cultivation studies share substantial overlap with taxon lists generated using more modern techniques, such as 16S rDNA amplicon sequencing and metagenomics [14–16]. A much more recent study, which compared large-scale anaerobic culturing to direct molecular ecology-based community enumerations, also supports the idea that most human gut bacteria can be readily grown outside of the host [17]. Taken together, these observations reinforce the imperative for continued culturing of microorganisms from the human gut so that their physiology, both alone and in communities, can be studied and understood in great depth [18].

Bacteriological studies into the abilities of human gut bacteria to degrade dietary fiber and mucin polysaccharides did not initiate in earnest until the 1970s, catalyzed by the seminal efforts of Salyers, Wilkins and colleagues, which involved fermentation studies on a large collection of cultured human gut bacteria [15,16]. Such analyses continue to be extended to a range of polysaccharides, as well as oligosaccharide components accessible through specific enzymatic treatment and fractionation [19–23]. These *in vitro* surveys have highlighted that the Bacteroidetes possess notably broad abilities to digest a diverse array of mostly soluble polysaccharides. In contrast, more recent work by Flint and co-workers has suggested that members of the Firmicutes, which demonstrated more limited catabolic breadth in early studies, possess the ability to attack more insoluble substrates that are characteristic of the natural plant fibers in our diets and may serve as “keystone” polysaccharide degraders [24,25].

Moving beyond descriptive growth studies, several researchers have provided molecular insight into the enzyme-based strategies through which human gut bacteria process complex carbohydrates, such as starch, inulin and many other polysaccharides [26–31]. These paradigms extend to various Gram-negative and Gram-positive bacteria and have provided a framework for understanding the molecular processes involved. Still, however, numerous questions remain, including (i) which species compete most efficiently for each available polysaccharide, (ii) to what extent and how do the species present cooperate during polysaccharide degradation and (iii) are dominant rumen bacterial strategies, such as deployment of cellulosomes by Gram-positive species, at work in the human colon? *An additional question that serves as a central focus for this review is how finely tuned are the relationships between members of our gut microbiota and the myriad chemical differences present in the polysaccharides that are so important to their biology?*