Spotlight

The Enigmatic Universe of the Herbivore Gut

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The herbivore gut is a fascinating ecosystem exquisitely adapted to plant biomass degradation. Within this ecosystem, anaerobic fungi invade biomass and secrete hydrolytic enzymes. In a recent study, Solomon *et al.* characterized three anaerobic fungi by transcriptomics, proteomics, and functional analyses to identify novel components essential for plant biomass deconstruction.

Strictly anaerobic fungi are an unusual group of zoosporic fungi that are a relative rarity in natural ecosystems, yet they are common in the gut of various herbivores (ruminants and large monogastric herbivores) [1]. In these environments, anaerobic fungi are elegantly tailored for the deconstruction of the food these animals eat: plant biomass. The anaerobic fungi found in the herbivore gut are members of a basal group of fungi (Neocallimastigomycota) and are characterized by forming zoospores (motile asexual spores) and rhizoids (Figure 1). They also lack mitochondria but contain hydrogenosomes, where ATP is synthesized [2]. In the environment of the gut, these anaerobic fungi partner with methanogenic archaea to synergistically deconstruct lignocellulose. The archaea utilize fungal metabolic byproducts that could be potentially inhibitory to fungal metabolism (hydrogen and carbon dioxide) to produce methane, thus enhancing fungal deconstruction of plant biomass.

The synergistic relation between anaerobic fungi and archaea in the gut of herbivores enables the reproduction of these microorganisms and also provides simple carbon and nitrogen compounds that are absorbed by the host [3]. Although anaerobic fungi comprise only a fraction of the gut microbiome in herbivores (~8%), they are believed to be important players in the deconstruction of plant biomass, through a combination of enzymatic deconstruction and physical disruption via invasive growth of the rhizoids [2] (Figure 1). However, unlike many of the prokaryotic species identified in the herbivore gut by metagenomic analyses [4], most of these anaerobic fungi are cultivatable. This experimental tractability led to the recent study by Solomon *et al.* [5], which focused on the characterization of three members of the Neocallimastogomycota isolated from feces from a horse (*Piromyces finnis*), a goat (*Neocallimastix californiae*), and a sheep (*Anaeromyces robustus*). These three anaerobic fungi showed robust activity towards both cellulosic and hemicellulosic substrates.

The transcriptomes of these gut fungi were analyzed under different growth conditions and revealed that they have a large and comprehensive array of biomass-degrading enzymes, including genes encoding proteins predicted to be involved in carbohydrate modification and utilization (so-called CAZymes) [5]. Transcriptional analyses showed that 2% of the transcripts from anaerobic fungi under lignocellulolytic conditions encoded predicted CAZymes; further proteomic analyses confirmed secretion of an array of hydrolytic enzymes across 25 glucosyl hydrolase families [5]. Interestingly, *Piromyces* showed expansions of several CAZyme family members, including GH10, predicted to be involved in hemicellulose deconstruction, the β-glucosidase GH5 family, the endoglucanase GH45 family, and polysaccharide deacetylases. This expansion in genes encoding CAZymes was also reported via genome analysis of another member of the Neocallimastigomycota, *Orpinomyces* sp. [6]. This expansion of genes encoding CAZymes in anaerobic fungi has been hypothesized to be partially responsible for the impressive hydrolytic capacities of these organisms. Future comparisons between *Orpinomyces sp.* [6] and the anaerobic fungi characterized in this study will be informative on conserved and divergent functions involved in lignocellulose degradation and adaptation of anaerobic fungi to the herbivore gut.

Transcriptional analyses of gene expression of *Piromyces* under different carbon regimes also revealed that genes encoding CAZymes were differentially regulated under growth conditions in simple carbon sources (i.e., glucose) versus complex biomass [5]. This fact was utilized to identify potentially novel genes encoding biomass-degrading proteins via co-clustering analyses with genes encoding predicted CAZymes that also displayed differential co-regulation. Transcriptome analyses also revealed an unexpected potential role of noncoding antisense RNA in regulating CAZyme production. Long noncoding RNAs, which were antisense for transcripts encoding CAZymes, were enriched for this functional class, in addition to other functional categories that included genes encoding enzymes involved in more basic cellular functions, such as ribosome biogenesis and RNA-dependent DNA replication. Interestingly, the production of antisense RNA during plant biomass utilization was also reported in the filamentous fungus *Aspergillus niger* [7]. These data suggest that fungi have evolved additional, potentially global, regulatory features for the production of hydrolytic enzymes that are associated with plant biomass utilization.

The lignocellulolytic arsenal of anaerobic fungi comprises secreted enzymes, as well as extracellular multienzyme complexes called 'cellulosomes' [8]. Cellulosomes were first identified in the bacterial family Clostridiaceae [9]. These large multienzyme complexes link diverse enzymes that function synergistically for high-efficiency plant biomass degradation (Figure 1); this efficiency stems from enzyme targeting via carbohydrate-binding modules (CBM), as well as the relative organization of the

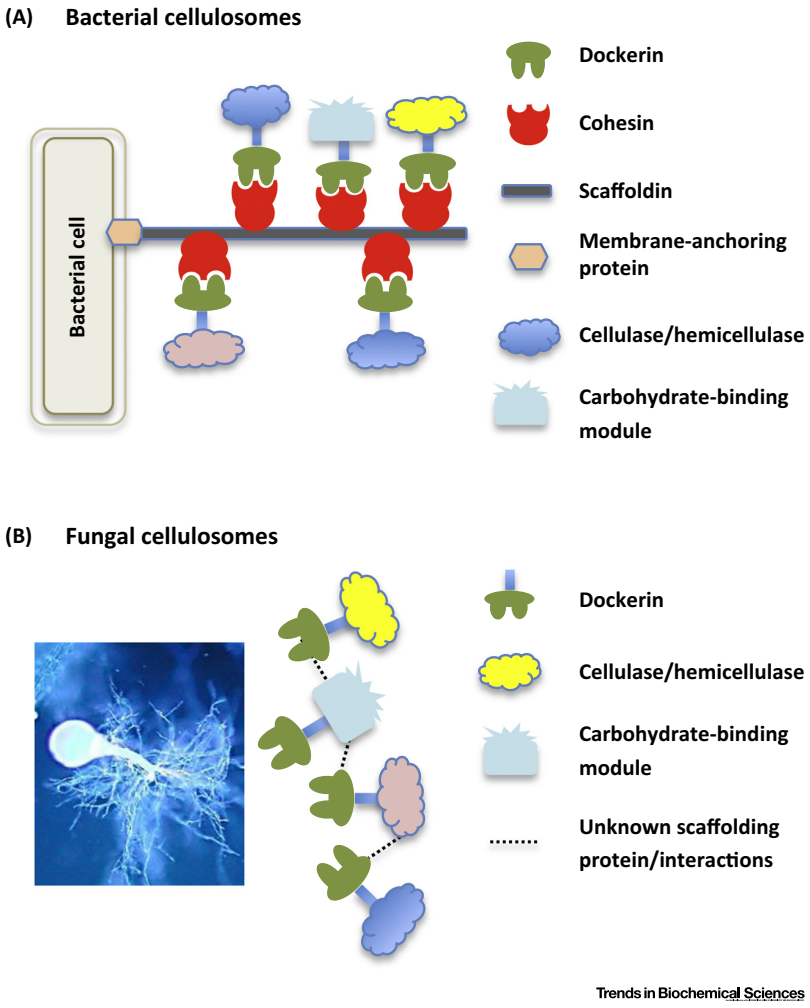

Figure 1. Comparison of the Organization of Cellulosomes from (A) Bacteria versus (B) Anaerobic Fungi. In bacteria, the cellulase and hemicellulase enzymes and proteins containing carbohydrate-binding modules (CBMs) are complexed to a scaffold protein (scaffoldin) via interactions between dockerin and cohesin molecules. The cellulosome complex is often tethered to the bacterial cell surface [9]. In anaerobic fungi, cellulases, hemicellulases, and CBMs can be complexed to dockerin proteins. A scaffolding protein has not been conclusively identified in anaerobic fungi; neither it is known how they may be associated with the fungal cell wall [3,5]. Micrograph of typical growth habit of anaerobic fungi, including rhizoids. Reproduced from https://anaerobicfunginetwork.wordpress.com/.

enzymes within the complex. In the study by Solomon *et al*. [5], the majority of the predicted lignocellulolytic enzymes in the three anaerobic fungal species contained a dockerin domain, similar to what was reported with *Orpinomyces* sp. [6]. In bacterial cellulosomes, dockerins are involved in the assembly of the cellulosome into higher order structures. Cellulosomes in bacteria are well characterized and have led to the development of 'designer' cellulosomes tailored for efficient plant cell wall deconstruction [10]. Although the molecular structure, architecture, and enzyme tethering of fungal cellulosomes produced by anaerobic fungi is not well characterized, the possibility of constructing synthetic 'designer' fungal cellulosomes will complement the work on bacterial cellulosome and may offer advantages to develop enzyme complexes and mixtures that will allow synergistic deconstruction of plant biomass. The work presented in the study by Solomon *et al.* [5] is a step towards achieving that goal.

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Spotlight

Clients Place Unique Functional Constraints on Hsp90

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Heat shock protein 90 kDa (Hsp90) is required for the activation and stabilization of numerous client proteins, but the functional requirements of individual clients remain