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Lignocellulose deconstruction in the biosphere

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Microorganisms have evolved different and yet complementary mechanisms to degrade biomass in the biosphere. The chemical biology of lignocellulose deconstruction is a complex and intricate process that appears to vary in response to specific ecosystems. These microorganisms rely on simple to complex arrangements of glycoside hydrolases to conduct most of these polysaccharide depolymerization reactions and also, as discovered more recently, oxidative mechanisms via lytic polysaccharide monooxygenases or non-enzymatic Fenton reactions which are used to enhance deconstruction. It is now clear that these deconstruction mechanisms are often more efficient in the presence of the microorganisms. In general, a major fraction of the total plant biomass deconstruction in the biosphere results from the action of various microorganisms, primarily aerobic bacteria and fungi, as well as a variety of anaerobic bacteria. Beyond carbon recycling, specialized microorganisms interact with plants to manage nitrogen in the biosphere. Understanding the interplay between these organisms within or across ecosystems is crucial to further our grasp of chemical recycling in the biosphere and also enables optimization of the burgeoning plant-based bioeconomy.

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Introduction

Photosynthesis and the resulting plant biomass is the only significant source of organic compounds in the terrestrial biosphere. The primary product of photosynthesis, cellulosic biomass, has evolved to be recalcitrant to deconstruction by microorganisms and their enzymes. This recalcitrance is due to natural barriers in plant mesostructure (bark, rind, and vascular networks); as well as the composition, structure, and chemical linkages in the plant cell wall. Cellulose crystallinity can be itself a barrier to enzymatic deconstruction, but the complexity and heterogeneity of the xylan matrix covering microfibrils further restricts enzyme accessibly and requires a large suite of xylan degrading enzymes [1,2]. Finally, lignification of the plant cell wall that provides rigidity to the plant is an impediment to efficient deconstruction by further reducing accessibly.

To overcome this natural recalcitrance, fungi and bacteria have developed a diverse set of enzymes and strategies suited for the ecosystem in which they occur. These strategies are primarily based on the use of glycoside hydrolases (GHs) (more than 140 GH families to date) [2]. Additionally, some fungi and bacteria can deploy oxidative processes that assist GHs in the deconstruction of biomass [3]. These enzymes are efficient enough for the microorganisms to grow on biomass as their sole carbon source, but have rather low turnover rates compared to other enzymes. Additionally, they are often more efficient in the presence of the microbe that produces them [4].

Biomass degrading microbes also rely on inter-microbial synergy to thrive in their natural environment where these interactions depend on the composition of microbial communities and the specific environmental conditions encountered. Moreover, these interactions can be crucial to the survival of these microorganisms and represent a vast resource of knowledge that can help us understand the chemical biology of carbon/nitrogen recycling and biomass deconstruction in the biosphere.

Plant cell wall structure

Plant biomass is composed of several energy-rich biopolymers that are arranged into a hierarchical structure to form the fiber reinforced matrix of plant cell walls. This material, termed lignocellulose, displays impressive structural complexity and robust functionality. During the lifetime of the plant, specialized cells in plant stems provide physical support and also form the conduits through which water and nutrients are transported. The mature cell walls in these supportive and conductive tissues typically comprise three ultrastructural domains: the middle lamella, the primary wall, and the secondary wall. The middle lamella of vascular cells

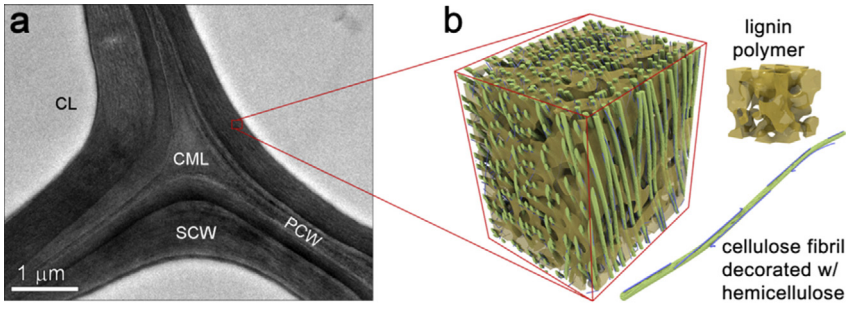
is heavily lignified and serves to adhere neighboring cells. The primary wall is the first layer of the wall to be synthesized during plant growth and consists of several layers of differently oriented cellulose microfibrils [1]. The secondary wall is synthesized after the primary wall is completed and provides substantial mechanical reinforcement to the vascular tissue. The secondary wall is distinct from the primary wall in that it is synthesized by the individual cell that it encapsulates whereas synthesis of the primary wall is achieved jointly by both cells that boarder the wall. By the time the secondary cell wall has been produced and the cell wall has lignified it is difficult to delineate the primary cell wall from the middle lamella. The term compound middle lamella (CML) is used to refer to these two layers collectively (Figure 1a).

Although the precise architectural details of lignocellulose nanostructure vary among plant species and tissues and remain an active area of research, some general agreement exists and informs future studies of efficient plant deconstruction. Aggregates of cellulose chains form strong and highly ordered bundles of cellulose micro-fibrils and macrofibrils [5], which serve as the rigid scaffolding structure and are deposited in discrete layers or lamella in the cell wall. These cellulose fibrils are decorated and interconnected with hemicellulose, which is a structurally diverse, branched polymer composed of various sugars including xylose, arabinose and mannose (Figure 1b). In the case of cells that produce a secondary cell wall, lignin, an amorphous polymer of different phenylpropanoid units, fills much of the remaining void volume of the cell wall [6]. Lignin provides additional mechanical strength to the composite and increases the hydrophobicity of the walls to aid in transport of water. In addition, lignin serves as a defense mechanism to prevent deconstruction by the hydrolytic enzymes secreted by pathogens. In most land plants, most of the cellulose is found in such lignified secondary cell walls, which poses a considerable challenge to biochemical deconstruction.

Hydrolytic and oxidative mechanisms of enzymatic cell wall deconstruction

In Nature, bacteria and fungi commonly deconstruct biomass by producing and secreting a combination of synergistically acting enzymes [7••]. The most abundant enzymes in these mixtures are hydrolytic glycoside hydrolases (GHs) and carbohydrate esterases. Other less abundant enzymes include polysaccharide lyases, 'auxiliary activity' enzymes (AA) [2], and cellodextrin phosphorylases. In the system used to classify carbohydrate active enzymes based on sequence and structure (CAZy), the GHs are represented by more than 140 different families [2]. Based on their mechanism and role in lignocellulose deconstruction there are three main classes of GHs, exoglucanases, endoglucanases, and cellobiases. Exoglucanases are processive enzymes and can cleave a cellulose polymer from either the reducing or non-reducing end of the polysaccharide chain. Endoglucanases typically hydrolyze cellulose chains nonprocessively anywhere along the polysaccharide chain. However in some cases endoglucanases can be processive exhibiting high cellulolytic activity [8–10]. Cellobiases primarily hydrolyze the cellobiose dimer into glucose monomers. These GHs cleave glycosidic bonds using one of two different types of catalytic mechanisms: Firstly, inverting, (i.e. inversion of anomeric configuration), wherein the catalytic acid and base residues generally achieve hydrolysis in a one-step mechanism [11,12]; or secondly, retaining, (i.e. retaining of anomeric configuration), wherein there is a general acid/base residue and a potential nucleophile used to conduct a Koshland type hydrolysis mechanism [13]. In this two-step mechanism, the first step is glycosylation (formation of a glycosyl enzyme intermediate) and the second step is deglycosylation (the glycosyl enzyme is hydrolyzed by water). The diversity of these GHs represents a vast arsenal of specific activities for the efficient deconstruction of biomass in the biosphere. However, microorganisms have also evolved ways to increase substrate specificity and enzyme kinetics by physically linking polysaccharidases in close proximity, increasing efficiency.

Figure 1



(**a**) Transmission electron micrograph of cell walls from vascular tissue in maize. CL, cell lumen; CML, compound middle lamella; SCW, secondary cell wall; PCW, primary cell wall. (**b**) Depiction of the structure of the lignocellulose composite in secondary cell walls.