Microbial extracellular enzymes and natural and synthetic polymer degradation in soil: current research and future prospects

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Abstract
Bacteria and fungi encounter complex organic matter in soil that is a potential source of the energy, carbon, and nutrients that are required for cell maintenance and growth. Cellulose and lignin are two of the most abundant biopolymers in detritus. However, bacteria and fungi do not have the ability to transport these macromolecules into the cytoplasm. Instead they depend on the activity of extracellular enzymes which generate soluble low molecular mass compounds that are recognized by cell wall receptors and transported into the cell. Many organic pollutants in soil are polymeric and poorly soluble (e.g. PAHs, PCBs) or toxic and these also require extracellular catalysis prior to uptake and metabolism. The complexity and diversity of extracellular enzymes and the macromolecules that they degrade will be reviewed and the many locations and multiple fates of these enzymes once they have left the cytoplasm illustrated. The many ways in which extracellular enzymes overcome the destructive or inhibitory properties of the soil matrix and the various strategies that microbes adopt for effective substrate detection and utilization will be described.

Key Words
Extracellular enzymes; cellulose; lignin; xenobiotics, microbial ecology**.**

Introduction

Enzyme synthesis and secretion is an energetically expensive process and regulatory mechanisms and ecological strategies have evolved to ensure the efficient capture of the products of substrate catalysis. However, soil is a hostile environment for extracellular enzymes because, once they leave the cell, they are subject to denaturation, degradation, adsorption and dilution. The locations and functions of enzymes in soil have been researched and discussed for decades (Burns 1978; Burns 1982; Caldwell 2005; Nannipieri *et al*. 2002; Wallenstein and Weintraub 2008) but recent advances in molecular, microscopic, and analytical techniques coupled with imaginative thinking (Allison 2005; Bouws *et al*. 2008; Wallenstein and Weintraub 2008) have begun to provide new insights. Developments have been motivated by the need to understand how the activities of enzymes contribute to a large number of industrial, medical and environmental processes.

Substrate and enzyme diversity in soils

Extracellular enzymes in soils catalyse the degradation of plant, animal and microbial macromolecules in addition to many potentially polluting xenobiotics. The most common plant polymers, cellulose and lignin, depend on the simultaneous and/or sequential activities of a large number of enzymes produced by a diverse community of bacteria and fungi; it is likely that more than fifty different extracellular enzymes are involved in the breakdown of a plant leaf prior to the low molecular mass carbon products entering the cell. Basidiomycete and ascomycete fungi are major degraders of cellulose, employing a battery of extracellular hydrolytic enzymes including endo-1,4-β- glucanases, cellobiohydrolases and β-glucosidases. The best known cellulose degrader, *Trichoderma reesei*, has thirty or more glycosyl hydrolases including seven endo-glucanases, and a secretome containing greater than one hundred proteins. Lignin, with which cellulose is usually associated, is a chemically complex phenylpropanoid that is degraded by a suite of oxidative enzymes containing laccases, manganese peroxidases and lignin peroxidases (Baldrian 2006; Osono 2007). The involvement of Fenton chemistry in the process demands the input of enzymes generating hydrogen peroxide as well as Fe^{2+} and Mn^{2+}. The white rot fungus, *Phanerochaete chrysosporium,* has more than 85 genes for glycosyl hydrolases, in excess of 100 for 'ligninases' and a secretome of almost 800 proteins. The feasibility of using *P. chrysosporium* (and many other fungi) for the oxidative degradation of organic pollutants, such as PAHs, PCPs, dioxins and many pesticides has been much studied (Rubilar *et al*. 2008; Husain *et al*. 2009).

Regulation, location and ecology of extracellular enzymes

In some cases, microbes produce small amounts of extracellular enzymes, regardless of substrate availability,

as a mechanism to detect substrate. If the substrate is present, these constitutive enzymes generate signals that induce additional enzyme synthesis. The synthesis *de novo* of many celluloytic extracellular enzymes is stimulated only in the presence (or sometimes absence) of a suitable substrate or other inducer whereas, in contrast, many 'ligininases' respond to stress factors such as redox potential, ionic strength, Fe^{2+}, CO_2, light, sulphide and sulfate and oxalic acid.

In broad terms, extracellular enzymes are contained within the periplasmic space (Gram-negative bacteria), associated with the outer cell wall, or released into the soil. However, the multiple functional locations of extracellular enzymes, combined with the capacity of the microbial community to detect potential substrates, suggest that there are many ways in which macromolecular organics can be transformed into soluble matter.

Enzymes that are retained on the cell wall are likely to be configured such that their active sites are exposed and the zones that are susceptible to attack by proteases are protected. Other cell-bound extracellular enzymes include those contained within a multicellular biofilm and others that are protected by specialized structures attached to the cell wall. The latter are the cellulosomes and contain cellulases (as well as hemicellulases and pectinases) arranged on a scaffold that facilitates efficient cleavage of polysaccharides (Bayer *et al*. 2008). Cells with bound enzymes must be in contact with their substrate.
Once enzymes have diffused away from their parent cell they may be sorbed, denatured and degraded. However, some extracellular enzymes are more stable than their intracellular counterparts because they are glycosylated, have disulfide bonds: modifications providing thermo-stability, a broad pH range for activity, and some resistance to proteases. In addition, some enzymes become stabilized through interactions with clay minerals and humic acid and retain a proportion of their activity (Allison 2006; Quiquampoix and Burns 2007). Stabilized soil enzymes represent a reservoir of potential activity and may represent the first catalytic response to changes in substrate availability in soils as well as serving as the originator of signal molecules for the microbial community. Even if the enzymes survive, the substrate may not be found and, even if it is, the correct combination of enzymes in the right sequence must be present for catalysis to occur.

A way to overcome some of these constraints is suggested by a mechanism that involves microbes 'sensing' both the substrate and their own population numbers. In this way gene function is connected to cell density and enzymes are only synthesizing and/or secreted when cell numbers are high enough to have a major impact. This is a process known as quorum sensing and has been well-described for many phytopathogens especially *Erwinia* species (Barnard and Salmon 2007). Quorum sensing in the rhizosphere is believed to be an important controlling process for all sorts of microbial interactions (DeAngelis *et al*. 2008).

Once in contact with their substrate many polysaccharases have a number of ways in which they not only maintain their stability but also increase their activity. One mechanism relates to the all-important substrate binding moiety (Wilson 2008) which, in the case of cellulase, anchors the enzyme to the substrate at appropriate points for the enzyme's catalytic domain to cleave the β-1,4-linkages. Few microorganisms secrete all the necessary enzymes and must rely on other microbes to successfully generate the soluble products. This observation reinforces the idea of a community-driven process.

However they are generated, the products of extracellular organic matter breakdown may be intercepted by other microbes which, although not investing any resources in enzyme production, will benefit (Allison 2005). Some microbes employ antibiotics and enzymes to reduce this 'cheating', others rely on the activities of predators to control their rivals. Of course, what might appear as cheating may be part of a complex and poorly understood microbial community synergy: the so-called cheaters provide some direct or indirect benefit to the cheated. Or it may be that the benefits of a successful extracellular depolymerization far outweigh the disadvantages derived from some of the products being high-jacked.

Conclusion
An enhanced knowledge of extracellular enzyme function will have many practical applications, including manipulating the soil for bioremediation, biocontrol, plant nutrient generation and availability, aggregate stability, and C cycling and sequestration. There are also implications for plant pathology, food quality and storage, biofuel production, and the impacts of climate change on enzyme activities and the humic matter pool. One of the greatest challenges in soil biology is to link the functional and ecological aspects of microbial extracellular enzyme activities to organic matter degradation. We are now equipped with the analytical (electrophoretic, chromatographic, mass spectrometric), microscopical (fluorescence, scanning probe, atomic and ultrasonic force, confocal laser, differential interference), molecular (genomics,

proteomics, metabolomics, secretomics, metagenomics) and bioinformatic tools to achieve these objectives (Wallenstein and Weintraub 2008). Are the activities of microbial enzymes in soil an example of organized chaos, ongoing selection processes or the expression of an advanced and stable degradative community? The next few years will answer many of these questions.

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