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PREFACE

# Exploring and integrating cellulolytic systems of insects to advance biofuel technology

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In line with the requirements for sustainable economies and clean environments, cellulose-based biofuels have recently received tremendous attention both in industry and academic communities worldwide. Alternative and renewable fuels derived from lignocellulosic biomass offer the potential to reduce our dependence on fossil fuels and mitigate global climate change. The world, therefore, is on the verge of an unprecedented increase in the production and use of biofuels. However, in industry, breakthrough technologies to overcome barriers to developing cost-effective processes for converting biomass to fuels and chemicals are not yet fully realized. It is worthy to note that, over the past two decades, industrial bioethanol technology has mainly been based on biocatalysis and fermentation technologies from bacterial and fungal cellulolytic systems, in combination with breakthroughs in molecular genetics, enzyme engineering and metabolic engineering. In practice, the current state of technology with respect to biomass conversion is still far away from being mature for large scale application due to its efficiency and processing economics. To improve upon our current technology, it seems that we need to review our ongoing strategy and explore/learn from other sound cellulolytic systems in nature, such as wood-feeding termites or other insects. Such insects can process lignocellulosic biomass much more efficiently with their highly specialized gut systems, which can truly be considered as highly efficient natural bioreactors.

With some of the most intractable issues facing the world regarding efficient and economic conversion of cellulosic biomass, this special publication comes at a critical and timely moment.

Most insects are unable to use lignocellulosic substrates as their main food sources, but some insects subsist on lignocellulosic biomass as their only foods. The types of biomass fed upon by cellulolytic insects range from agricultural crops to forest woody substrates, such as in the case of termites (all seven families), wood-feeding roaches (Blattidae, Cryptoceridae), beetles (Anobiidae, Buprestidae, Cerambycidae, Scarabaeidae), wood wasps (Siricidae), leaf-shredding aquatic insects (Pteronarcidae, Limnephilidae, Tipulidae), silverfish (Lepismatidae), leaf-cutting ants (Formicidae), and so on. Cellulose digestion has been demonstrated in more than 20 insect families representing ten distinct insect orders, for example, Thysanura, Plecoptera, Dictyoptera, Orthoptera, Isoptera, Coleoptera, Trichoptera, Hymenoptera, Phasmida and Diptera. The ability of these insects to feed on wood, foliage and detritus has recently stimulated extensive investigation into the mechanisms of how these insects digest the structural and recalcitrant lignocellulose in their foods, as well as their potential to advance current biofuel technologies and processing. Recent studies using advanced molecular biotechnologies, such as metagenomics, proteomics, transcriptomics, and so on, have brought new insights into the mechanisms of biomass deconstruction within these small, but complicated insect gut systems. It has been reported that the digestion efficiency of wood-feeding termites is 74%–99% for cellulose and 65%–87% for hemicellulose, which mainly function via a collaboration between two catalyst systems: (i) termite endogenous catalyst systems, and (ii) catalysts from a variety of gut symbiotic microorganisms, including cellulolytic protozoa and bacteria. The number of the novel cellulases and hemicellulases, as well as the associated encoding genes from a variety of cellulose-feeding insects has been continuously updated in recent years. Descriptions of lignase enzymes and the genes that code them have been lacking; however, recent findings have suggested several viable candidates in both areas. Screening of the genes/enzymes showing suitable properties for industrial applications is one of the important applied goals behind the exploration of insect cellulolytic systems. Besides unveiling the mystery of the insect catalyst systems on cellulosic substrates, studies on physiochemical microhabitats of insect gut systems have also shed new light on better understanding of what types of gut environments may actually support an efficient cellulolytic system. Clearly, all these investigations are theoretically and practically substantial for understanding insect catalyst systems and advancing current biofuel technology. To facilitate the integration of these two different areas, this special issue bridges the gap between investigations of insect cellulolytic systems and their potential applications for biofuel refinery operations. This issue, therefore, covers a range of recent experimental advancements with seven original research papers and five review papers that address the current state of the art in research, methodology and application, as well as various insect cellulolytic systems.

Critical to the efficiency of biomass processing in the biofuel industry are pretreatment technologies that are developed for a variety of the diverse feedstocks that are currently available. Pretreatment regimes must be designed to remove substrate-specific barriers to cellulases to improve cellulose digestion. A review article by Scharf and Boucias (2010) reviews recent findings from research into the host termite transcriptome that have revealed candidate enzymes (lignases and phenolic acid esterases) to modify lignin components from lignocellulosic substrate; and they also discuss research needs and opportunities for consideration by entomologists working in this area. Furthermore, Ke *et al*. (2010) report on oxygen profiling *in situ* within the fore-, mid- and hindgut of two wood-feeding lower termite species (characterized by the presence of symbiotic protists residing in hindgut) and also confirm that lignin modification/disruption mainly occurs within termite fore- and midgut compartments, after which the wood particles then move to the hindgut for further depolymerization by protozoa residing in hindgut. This preconditioning processing on lignin components likely permits greater access to cellulose.

With regard to developing novel cellulase biocatalysts based on genes discovered from wood-feeding lower termites, a paper by Zhang *et al*. (2010) describes two recombinant endogenous glycosyl hydrolases from a lower termite species, expressed in *Escherichia coli* that functionally convert cellulose to glucose. This work will surely help scientists optimize recombinant cellulolytic enzyme production and combinations for biomass conversion. Cao *et al*. (2010) report on examinations of hydrogen/methane emission from three lower termite species as byproducts produced during the course of cellulose degradation. These findings imply a unique mechanism for producing biohydrogen as a by-product during cellulose conversion through gut cellulolytic and metabolic systems. To better understand the symbiotic composition differences between gut and nest-associated microbial communities in fungus-growing termites (a higher termite species), Long *et al*. (2010) report differences in composition for both fungal and bacterial communities between two different symbiotic microhabitats. The paper by Long *et al.* (2010) contributes to a better understanding of the potentially integrated functions played by in-gut symbionts and external "ecto"-symbionts during the wood degradation process.

Apart from the wood-feeding termites, Geib *et al*. (2010) report on enzyme biochemistry in a wood-feeding beetle, the Asian longhorn beetle, relevant to lignocellulose degradation. Geib *et al*. (2010) used zymogram analysis to identify and characterize cellulases and hemicellulases active against cellulose and hemicellulose substrates. Because this beetle feeds on a range of tree species and uses them as sole food sources, mining this insect gut for lignocellulases can potentially yield new enzymes for processing lignocellulolytic material into cellulosic biofuels. For another cellulose-consuming beetle species, Huang *et al*. (2010) review the physiochemical properties of the scarab beetle gut (larval stage), the diversity and digestive roles that symbiotic microflora play in the scarab gut, and they further discuss the potential for applying these digestive processes in artificial bioreactors.

Exploring another specific cellulose-consuming insect from the order Diptera (crane fly), which is a leaf shredding aquatic insect that lives in forested ecosystems, Rogers and Doran-Peterson (2010) report on the analysis of cellulolytic and hemicellulolytic enzyme activity within this insect gut (larval stage). Rogers and Doran-Peterson (2010) also report on identification and characterization of a novel cellulolytic bacterial species isolated from its gut system. In a related report from the same laboratory, Cook and Doran-Peterson (2010) show the potential of the crane fly gut to serve as a natural biorefinery model to apply in improving and developing biomass-to-biofuel technology.

The advancement of genomics and proteomics research tools are expected to allow new insights into the mechanisms for wood deconstruction by cellulose-feeding insects, as well as facilitate the discovery of new cellulolytic enzymes from a wide range of cellulolytic systems. On this topic, Willis *et al*. (2010) review the diverse methodologies used to detect, quantify, clone and express cellulolytic enzymes from insects, as well as their advantages and limitations. In addition, Shi *et al*. (2010) also provide a comprehensive review on molecular approaches to study insect gut symbiotic microbiota with a variety of "omics" tools. Last, the contribution by Landis and Werling (2010) addresses pest management and landscape ecology issues in relation to biofuel crop production; specifically, potential arthropod community responses to a large scale production of biofuel crops and biomass harvesting from North American forests. In this review paper, some critical topics relevant to the arthropod ecology in impacted systems are also proposed to meet the coming challenges associated with a large-scale planting of energy crops and forest biomass harvesting. This is an important emerging area of research stemming from renewed interests in biofuels that is creating opportunities for entomologists.

In summary, this special issue focuses on broad-ranging areas of progress and challenges associated with the utilization of gene, catalyst, and other unique mechanisms from wood-feeding insects, as well as ecological impacts and pest management needs of biofuel-based agriculture and forestry. This special issue comes at a time when government and industry are scaling up their investment in biofuels, with the expectation of a long-term need for alternatives to petroleum-based liquid fuels. Exploring insect cellulolytic systems will lead to the discovery of a variety of novel biocatalysts and genes that encode them, as well as associated unique mechanisms for efficient biomass conversion. This new and evolving multidisciplinary area has emerged between insect and bioengineering sciences; without question, it will pave the way for future breakthroughs and innovations in associated areas of industrial biotechnology. It is also hoped that this special issue can promote productive collaborations between scientists working in the various disciplines represented herein.

We hope that readers will find these articles interesting and helpful to their research efforts. It has been our pleasure to put together this special issue in *Insect Science*. We would like to thank Professor Le Kang, Editor-in-Chief, for providing researchers a unique forum in which to report ongoing research activities on insect cellulolytic systems and their potential to update the current technology for biomass processing. We would also like to sincerely thank Dr. Yun Xian Zhao for expert editorial assistance, and all of the authors that have contributed to this special issue for their dedicated efforts and excellent contributions. We acknowledge with appreciation the assistance of the reviewers who have given their valuable support and expertise in reviewing manuscripts submitted for this special issue. The quality of the issue can be attributed in large measure to the quality of their efforts, for which we are sincerely grateful.

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