Jizhong Zhou, J Fundam Renewable Energy Appl 2017, 7:3(Suppl)
http://dx.doi.org/10.4172/2090-4541-C1-025

Jizhong Zhou

University of Oklahoma, USA

Functional genomics analysis of *Clostridium cellulolyticum* for lignocellulose bioconversion

The lack of efficient genetic tools for targeted genome editing and transcriptional control hinders functional genomics studies and microbial engineering. Research in the model organism of mesophilic cellulolytic clostridia, *Clostridium cellulolyticum*, which is capable of one-step lignocellulose bioconversion, is facing the same challenge. Here, we successfully developed an efficient Cas9 nickase-based genome editing tool to modify the *C. cellulolyticum* genome. This tool not only successfully overcame the toxicity of previously reported severe DNA damage caused by Cas9-based editing methods, but also demonstrated the advantage of marker-independent gene delivery, versatile editing, and multiplex editing in a single step at a very high editing efficiency and specificity. The combinatorial method using the Cas9 nickase editing tool to chromosomally integrate RNA repression modules enabled us to stably manipulate essential metabolic genes in this bacterium in a plasmid-independent way. With this superior editing tool, we conducted comprehensive studies on cellulose-degrading cellulosomes and carbon catabolite regulation (CCR), aiming to increase our understanding of lignocellulose degradation and carbohydrate assimilation in this bacterium. First, we genetically identified three important cellulosomal components (Dpi, Cel48F endocellulase and Cel9E exoglucanase). Our results revealed that Dpi, as an effective cysteine protease inhibitor, protects indispensable cellulases from proteolysis, providing the first evidence showing the in situ importance of cellulosomal protease inhibitors in cellulose degradation. Second, chromosomal integration of promoters into the *cip-cel* operon which encodes major cellulose-degrading enzymes dramatically enhanced both exoglucanase and endoglucanase activities of isolated cellulosome complexes, and subsequently improved the hydrolysis of cellulose to soluble sugars. Third, *C. cellulolyticum* lost the sugar-transporting phosphotransferase system and catabolically exhibited a very mild reverse catabolite repression. Mutagenesis of the predicted CCR regulatory system, including *hprK, crh* and *ccpA*, showed that cellobiose assimilation was independent of CCR, but the utilization of monomers (both pentoses and hexoses) and insoluble cellulose were tightly associated with CCR. This study also provided the first genetic evidence to show the indispensability of the *crh* and *ccpA* genes in cellulose catabolism. The observed differential reliance of carbohydrate utilization on this reduced CCR was explained by our transcriptomic analysis. The aforementioned functional genomics analysis provides novel insights into sugar assimilation, cellulose degradation, and cellular metabolism in *C. cellulolyticum*. These discoveries will help microbial engineers to develop feasible strategies to improve lignocellulose bioconversion, which can be technically further facilitated by the advent of our robust Cas9 nickase-based genome editing tool.

Biography

Jizhong Zhou is a George Lynn Cross Research Professor, Presidential Professor in the Department of Microbiology and Plant Biology and Director of the Institute for Environmental Genomics, University of Oklahoma (OU) Norman, OK, an Adjunct Senior Scientist at Lawrence Berkeley National Laboratory and an Adjunct Professor at Tsinghua University, Beijing, China. His expertise is in microbial ecology and genomics with current research focused on: (i) molecular community ecology and metagenomics, particularly of terrestrial soils and groundwater ecosystems important to climate changes and environmental remediation, (ii) experimental evolution and functional genomics of microorganisms important to environment and bio-energy, (iii) pioneering development of high throughput metagenomic technologies, particularly functional gene arrays for biogeochemical, environmental, and ecological applications, and (iv) theoretical ecology, particularly network ecology.

jzhou@ou.edu