

Microbial Stress Tolerance for Biofuels

Systems Biology

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Preface

The development of sustainable and renewable biofuels has attracted growing interests with concerns on increased oil demands and a cleaner environment worldwide after decades of attempts since 1950s. Biofuels conversion from renewable biomass including lignocellulosic materials and agricultural residues is considered as the second generation of transportation biofuels. The success of a bio-based economy requests not only the development of an appropriate infrastructure but also the meeting of significant technical challenges for a sustainable industry. The economy of fermentation-based bioprocess including bioethanol production relies extensively on the performance of fermentative microbes. It is vital to develop robust microbial strains for the next generation biocatalysts that are able to function under multiple stress conditions presented in the lignocellulosic biomass-based fermentation systems.

This volume is intended to provide a comprehensive study on microbial stress tolerance using a systems biology approach. It has no means to claim a complete coverage of all important aspects on such a complicated subject by this limited space. The urgency and necessity to address microbial stress tolerance in biofuels applications using comprehensive approaches of systems biology simply cannot be underestimated. In addition to outlining the most advanced knowledge in the respective fields, each chapter provides conclusive remarks and future perspectives intriguing active discussions and proposals. We are humbled to learn that a vast amount of unknown factors exist in detailed life events for microbial stress at the genome level. High levels of integrated interdisciplinary studies are expected to advance basic science on microbial stress tolerance and its applications of successful biofuels productions.

The book consists of two parts of topics around the subject. The first part, comprising the first eight chapters, covers advances and mechanisms of our current understanding on microbial stress tolerance. The second part, comprising the last four chapters, provides approaches and methodology recently developed in related fields with relevant application examples. In the section on advances and mechanisms, genomics aspects are first outlined for yeast tolerance and in situ

detoxification of inhibitory compounds generated during biomass pretreatment. New gene functions, multiple functions of a characterized gene, complex interplay, reprogrammed pathways, and overlapping gene regulatory networks are clearly involved in yeast tolerance at the genome level. A detailed discussion on genetics and regulation of glycogen and trehalose metabolism, important elements for yeast tolerance involved in glycolysis pathways and yeast function, are followed. The sophisticated regulatory system provides insight into yeast tolerance studies not only for nutrition supply but also significant for stressed physiological and toxic status. The next chapter describes molecular mechanisms of programmed cell death as a defensive function against acetic acid, another stress factor concerned about in biofuels fermentation process. Continued pursuing on signal transduction will further advance our understanding on the tolerance to acid stress conditions. The classic yeast *Saccharomyces cerevisiae* is a superb ethanol producing agent, yet it is sensitive to ethanol stress based on varied definitions. A comprehensive discussion on molecular mechanisms of ethanol tolerance by yeast is provided including new data obtained from comparable temporal dynamics of quantitative gene expression analyses. A prototype of molecular mechanisms on ethanol tolerance is presented. From the industrial bioprocessing point of view, the following chapter touches various stress conditions regarding high gravity ethanol fermentation. Bioprocess engineering appears to be a significant component that cannot be overlooked for large scale productions given multiple stress conditions recognized by lignocellulosic biomass conversion. Yeast tolerance has been observed to be closely related to balanced sugar utilizations. An inevitable topic on improving divergent biomass sugar utilization by engineered *S. cerevisiae* is attended in the next chapter. New strategies to improve xylose uptake and utilization by the yeast using synthesized genes and heterologous xylose transporter genes are presented. This advance is expected to lead flourishes of desirable second generation biocatalyst development. These chapters conclude the main coverage on ethanologenic yeast. For bacteria, genomics approaches on tolerance to biomass pretreatment inhibitors by ethanologenic bacterium *Zymomonas mobilis* are summarized. Accurate annotation strategy of the bacterial genome resulted in discoveries of new genes and functions contributing to acetate stress tolerance. Case studies of selected genes involving tolerance and a paradigm of strain development are discussed. In understanding microbial physiology of biofuels production, mechanisms and applications of microbial solvent tolerance are comprehensively addressed in a wide range of bacterial species as well as yeast.

The section on approaches and methodology starts with metabolic engineering using bacterial host for biofuels production in the light of stress tolerance controls. Concerns in developing biofuels producing agents regarding biosynthetic pathways and tolerance mechanisms are discussed with application examples. Such a tolerance is designed against both pretreatment inhibitors and toxic end products. At this end, robust strains encoded by chromosomal integration and free of antibiotic resistant markers are desirable. The next chapter describes basic principles and applications of metabolomics approaches including sample preparation, metabolomic analysis, identification and quantification of metabolites, data mining, and

biological interpretation for gas chromatography coupled to mass spectrometry (GC-MS) and liquid chromatography coupled to mass spectrometry (LC-MS) based strategies. A better understanding of metabolomics of microbial stress is expected to benefit optimization of biofuels fermentation processes. The following chapter introduces an automated plasmid-based functional proteomics system. The high throughput platform enables rapid clone and expression of heterologous genes for library screening and improved strain development. The closing chapter presents robust mRNA quantification references that can be used for unified and comparable gene expression data analyses under stress conditions. The fundamental biological process of gene expression raises useful phenotypes in mechanism studies of stress tolerance. Principles and applications as well as critical issues in unification of expression data analysis within and across different platforms of qRT-PCR array and microarray assays are discussed.

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