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Review

A comparative view of metabolite and substrate stress and tolerance in microbial bioprocessing: From biofuels and chemicals, to biocatalysis and bioremediation

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ABSTRACT

Metabolites, substrates and substrate impurities may be toxic to cells by damaging biological molecules, organelles, membranes or disrupting biological processes. Chemical stress is routinely encountered in bioprocessing to produce chemicals or fuels from renewable substrates, in whole-cell biocatalysis and bioremediation. Cells respond, adapt and may develop tolerance to chemicals by mechanisms only partially explored, especially for multiple simultaneous stresses. More is known about how cells respond to chemicals, but less about how to develop tolerant strains. Aiming to stimulate new metabolic engineering and synthetic-biology approaches for tolerant-strain development, this review takes a holistic, comparative and modular approach in bringing together the large literature on genes, programs, mechanisms, processes and molecules involved in chemical stress or imparting tolerance. These include stress proteins and transcription factors, efflux pumps, altered membrane composition, stress-adapted energy metabolism, chemical detoxification, and accumulation of small-molecule chaperons and compatible solutes. The modular organization (by chemicals, mechanism, organism, and methods used) imparts flexibility in exploring this complex literature, while comparative analyses point to hidden commonalities, such as an oxidative stress response underlying some solvent and carboxylic-acid stress. Successes involving one or a few genes, as well as global genomic approaches are reviewed with an eye to future developments that would engage novel genomic and systems-biology tools to create altered or semi-synthetic strains with superior tolerance characteristics for bioprocessing.

1. Introduction

1.1. Biofuels and chemicals from biomass, and the importance of strain tolerance to chemicals

The development of technologies for the production of fuels and chemicals from renewable biomass sources has been on the scientific and technological agenda of our nation for over 35 years, but never quite with the urgency of the last few years. Biomass is a carbon-neutral renewable resource for the production of biofuels and biologically produced chemicals and materials, thus leading to the widely-discussed concept of the biorefinery (Blanco-Rosete and Webb, 2008; Demirbas, 2009; Gibbons and Hughes, 2009; Rude and Schirmer, 2009; Yazdani and Gonzalez, 2007; Zheng et al., 2008). Although some success has been achieved thus far, scientific advances are expected to increase efficiency significantly, and there is increased optimism that cost-efficient production of biofuels (Stephanopoulos, 2007), materials and chemicals from lignocellulosic biomass will be widespread within 15 years. The list of chemicals that can be in principle produced from renewable resources is large and includes simple and complex carboxylic acids and alcohols, hydrocarbons, and diesel biofuels (Blanco-Rosete and Webb, 2008; Demirbas, 2009; Gibbons and Hughes, 2009; Rude and Schirmer, 2009; Yazdani and Gonzalez, 2007; Zheng et al., 2008).

So far, biofuel production has mainly focused on ethanol, which comprises 99% of the total biofuel consumption in the US (Rao et al., 2007). Butanol is also considered as an attractive biofuel, as it exhibits superior chemical properties in terms of energy content, volatility, and corrosiveness (Lee et al., 2008). Several other oxygenated organic molecules (such as non-fermentative alcohols, including i-butanol (Connor and Liao, 2009; Rude and Schirmer, 2009)) that can be produced from biomass can serve as biofuels, as well. Other biofuels include biologically produced hydrocarbons deriving either from isoprenoid or fatty-acid metabolism or microbial diesel fuels (Rude and

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Schirmer, 2009). Although it is still unclear if one or more such molecules will dominate markets in the future, the fundamental features of a biomass-to-biofuels or biomass-to-chemicals processes are common. Biomass is collected and treated to release the tangled lignin–cellulose fiber via ammonia explosion, weak acid boiling, or steam treatment (Hahn-Hagerdal et al., 2006). The suspension is then digested by cellulolytic enzymes that hydrolyze the hemicellulosic and cellulosic biomass to 5- and 6-carbon sugars, which can then be fermented by alcohol producing microorganisms such as *Escherichia coli* (Ingram et al., 1987), *Clostridium acetobutylicum* or other solventogenic *Clostridia* (Lee et al., 2008; Paredes et al., 2005), *Saccharomyces cerevisiae* (Rude and Schirmer, 2009), and *Zymomonas mobilis* (Stephanopoulos, 2007). Two common features of all such processes are: (1) the presence of molecules as a result of biomass pretreatment that may be inhibitory to the fermentation process for the production of the desirable molecule(s) and (2) the inhibitory nature of the desirable product (such as ethanol or butanol, other alcohols, hydrocarbons, succinate, butyrate and other carboxylic acids) or bioproducts. Accumulation of products during a fermentation, whether desirable or not, can be toxic to the biocatalysts, inhibiting cell growth or resulting in death. Similarly, toxic contaminants in biomass-hydrolysate substrates (Almeida et al., 2007; Martin et al., 2007; Rudolf et al., 2007) can inhibit cell growth and product formation. Inhibition from such chemicals typically limit product titers, affects fermentation performance and operational options (continuous vs. batch or fed-batch), and profoundly impacts process economics.

1.2. Bioremediation and whole-cell biocatalysis also benefit from tolerant strains

Bioremediation involves the conversion of toxic chemicals into benign or less toxic chemicals by biological means and typically by employing one organisms or a consortium of microorganisms (Bustard et al., 2000, 2002; Gupta et al., 2006; Pandey et al., 2009; Zhao and Poh, 2008). The effectiveness of these microorganisms in degrading toxic chemicals depends on their natural, selected or engineered tolerance to the chemicals present during the remediation process.

Whole-cell biocatalysis in two-phase systems containing an organic phase (typically solvent) has a broad spectrum of applications for the production of specialty or fine chemicals (Heipieper et al., 2007; Neumann et al., 2006; Sardessai and Bhosle, 2004). Two-phase systems are employed in order to solubilize reactants and/or products but also to deal with the problem of toxic substrates and/or products. The organic-solvent phase frequently serves as a means to reduce the concentration of a toxic chemical from the aqueous phase. The solvent used in these two-phase systems is typically somewhat soluble in the aqueous solution, and thus the organism or organisms employed must be tolerant to such solvents, as well.

In summary, from biorefinery and biofuel production to bioremediation and whole-cell biocatalysis, development of strains with superior tolerance characteristics to specific chemicals and general stressful bioprocess conditions is an important and widely recognized goal.

1.3. Chemical toxicity and tolerance are complex, multigenic phenotypes

As detailed below, tolerance of microorganisms to chemicals is a complex, multigenic trait and is affected by several process parameters such as pH, temperature, osmotic pressure, other small or large molecules, and pressure. Indeed, if one takes the core and most-widely examined problem of developing ethanol-tolerant strains, much effort has been based on the concept that ethanol (and more broadly solvent) tolerance can be controlled by a single gene, or a few genes. Yet, the tolerant phenotype is the result of a several simultaneous mechanisms of action, including molecular pumps, changes in membrane properties, changes in cell wall composition, altered energy metabolism, and changes in cell size and shape. These and related mechanisms are apparently independent from each other and involve genes or gene clusters widely dispersed on the chromosome or located on plasmids (Bernal et al., 2007; Isken and de Bont, 1998; Kivistik et al., 2006; Kobayashi et al., 2001; Neumann et al., 2005; Nikaido and Zgurskaya, 1999; Phoenix et al., 2003; Ramos et al., 2002, 1997; Volkers et al., 2006; Weber and de Bont, 1996; Wei et al., 2001).

1.4. Common chemicals and organisms encountered in biorefinery and biofuel bioprocesses

Typical or expected bioprocess-based metabolites include alcohols (e.g., ethanol, butanols and derivatized butanols, pentanols, hexanol, propanediol, butanediols), carboxylic acids (e.g., succinate, butyrate, acetate, propionate), aldehydes, ketones and hydrocarbons, and this list is ever expanding. The number of carbon substrates is also increasing as more complex carbohydrates from primary sources or from wastes are considered for the development of novel processes. They include the whole spectrum of 5- and 6-carbon sugars, glycerol, carboxylic acids (acetate, propionate), celluloses and xylans and their hydrolysates, starches, carbohydrates from corn or sugarcane refineries, and substrate impurities (such as byproducts from biomass hydrolysates). While most sugars and starches are not inhibitory to cells, most other substrates and substrate impurities can be toxic to cells.

Organisms employed in biorefinery and biofuel bioprocessing include yeast (*S. cerevisiae*, one of the major workhorses of modern biotechnology, but also other *Saccharomyces* strains, *Kluyveromyces marxianus*, *Pachysolen tanophilus*, *Sheffersomyces stipitis* (Gibbons and Hughes, 2009)) and other fungi (such as species in the genus *Aspergillus*, and *Gliocladium roseum* (Strobel et al., 2008), which is receiving attention recently in the context of microbial hydrocarbon production), *E. coli*, a major workhorse of modern biotechnology, and several other Gram-negative ($Gram^-$) bacteria such as *Z. mobilis* (Antoni et al., 2007), *Mannheimia succiniciproducens* and *Actinobacillus succinogenes* (Kim et al., 2007). The list of $Gram^-$ bacteria becomes much larger when considering applications in whole-cell biocatalysis and bioremediation, and would include organisms of the genus *Pseudomonas* such as *P. putida*. Among Gram-positive ($Gram^+$) bacteria, the list of species of importance to biorefinery and biofuel processes, as well as to biocatalysis, includes organisms in the genera *Bacilli*, *Clostridia* and *Lactobacilli*. Among $Gram^+$ organisms of importance to bioremediation processes are species of the genus *Deinococcus*, and notably *Deinococcus radiodurans*, which exhibits remarkable resistance to ionizing radiation (Cox and Battista, 2005) and is an essential microbe for remediation of sites contaminated with nuclear wastes.

2. Solvent stress, toxicity and tolerance

2.1. Classes of solvents

Solvents constitute a very wide class of molecules, both organic and inorganic. In the context of molecules produced by cells as useful or undesirable metabolites, or for applications in