222. Novel Mechanisms for Ionic Liquid Resistance in Microbial Biofuel Production

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http://www.jbei.org/research/divisions/deconstruction/microbial-communities

Project Goals: Natural microbial communities are rich sources of microbes, enzymes and transporters to improve the conversion of biomass to biofuels. The Microbial Communities group at the Joint BioEnergy Institute discovers these activities and translates them for applications in biofuel production.

Microorganisms have developed innovative ways to stay alive in extreme conditions, and many of these adaptive mechanisms operate to their advantage in anthropogenic milieux. Many forest soil microbes, for example, secrete highly active enzymes and can withstand fluctuations in temperature, pH, ionic strength, and hydration. Thus, using environmental bacteria could improve biomass degradation and reduce microbial growth inhibition from toxic byproducts. An alternative involves harnessing the positive traits of these native microbes to engineer existing laboratory strains for increased efficiency in cellulosic biofuel production.

We isolated a novel halotolerant bacterium, *Enterobacter lignolyticus*, from a tropical cloud forest soil community[1]. *E. lignolyticus* digests lignin[2] and moreover grows in the presence of relatively high concentrations of an ionic liquid used in pretreating biomass[3]. This chemical, 1-ethyl-3-methylimidazolium chloride, or [C_2mim]Cl, is an effective cellulose solvent but the imidazolium cation is especially toxic to biofuel producing microbes.

We examined changes in the global transcriptome of *E. lignolyticus* after exposure to [C_2mim]Cl, using RNA-seq transcriptomics to identify differentially expressed genes and the associated pathways[3]. In a parallel study we screened a library of *E. lignolyticus* genome fragments, and found an IL resistance system consisting of two adjacent genes[4]. One of these corresponded to the highest IL-induced transcript in *E. lignolyticus*. The two genes retain their full functionality when transferred to an *E. coli* biofuel host, with IL resistance established by an inner membrane transporter that is regulated by an IL inducible repressor. Expression of the transporter, a MFS multidrug efflux pump, is dynamically adjusted by a TetR-type regulator in direct response to IL concentration, enabling growth and biofuel production at levels of IL that are toxic to native strains (**Fig. 1**).

To further understand and overcome the growth inhibition caused by pre-treatment ionic liquids, we have isolated several other environmental microbes – Gram-negative and Gram-positive bacteria and fungi – that tolerate [C_2mim]Cl and [C_2mim][OAc], a more potent cellulose solvent. We identified other efflux pumps in both the MFS and SMR families that are effective in providing ionic liquid resistance (**Fig. 2**). The SMR pump genes occur in tandem, and some are adjacent to transcriptional regulators that may adjust pump levels in response to changing IL concentrations. Further studies are

aimed at fine-tuning these pumps and their regulators for use in biofuel-producing *E. coli* and *S. cerevisiae.* The discovery of IL-tolerant microbes and their resistance mechanisms will overcome a major hurdle in the use of these cellulose solvents, and will advance our progress towards readily and economically converting lignocellulose biomass to biofuels and renewable chemicals.

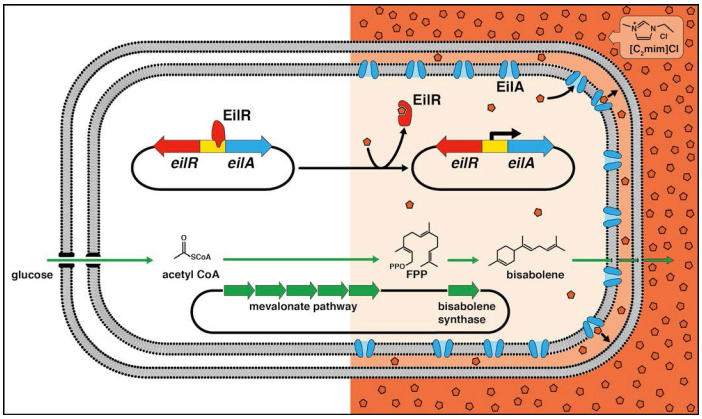
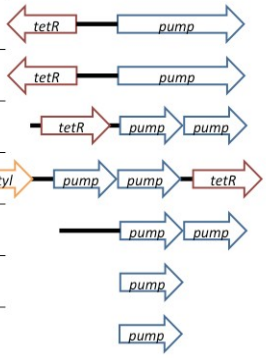


Figure 1 | Model of the IL tolerance mechanism engineered in a bacterium that converts hydrolyzed biomass into biofuel under two different conditions, either in the absence (left half) or presence (right half) of ionic liquid. *key*: bisabolene biosynthetic genes (green) and biofuel production pathway (green arrows), efflux pump EilA (blue), repressor EilR (red), intergenic region (yellow), imidazolium cation (orange pentagons).

Figure 2 | Library of resistance genes originating from several bacteria, including Salmonella and Bacillus strains, identified by screening genomic fragments in E. coli for growth in medium containing IL. Membrane transporters are either Multi- facilitator Superfamily (MFS) or Small Multidrug Resistance (SMR) efflux pumps, and some are associated with TetR-type regulatory genes. Although E. coli does not contain an MFS ortholog, two SMR homologs are present but only confer IL tolerance when expression is induced.

Origin	Pump	Regulator
E. lignolyticus	MFS	TetR
S. enterica	MFS	TetR
B. cereus	SMR pair	TetR
B. cereus	SMR pair	TetR & acetyl transferase
B. licheniformis	SMR pair	Putative riboswitch
E. coli	SMR	IPTG-inducible construct
E. coli	SMR	IPTG-inducible construct



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