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**Mechanism of Imidazolium Ionic Liquids Toxicity in *Saccharomyces cerevisiae* and Rational Engineering of a Tolerant, Xylose-Fermenting Strain**

Understanding how yeasts respond to ionic liquid solvents enables engineering of more efficient biocatalysts.

**The Science**

The method to deconstruct lignocellulosic biomass using ionic liquids (ILs) is a promising technology that can bypass expensive enzymatic pretreatments that are commonly used. A potential limitation to adoption of ILs for bioconversion is that residual IL solvent is toxic to microbes and can impede fermentation of biomass to biofuels. A technique called chemical genomics was used to identify yeast genes that confer sensitivity or resistance to IL, allowing researchers to identify a cellular substructure called the mitochondrion as the apparent target of IL toxicity. Targeted deletion of a gene identified from the chemical genomics screen in a xylose-fermenting strain of yeast greatly increased its IL tolerance, biomass-derived sugar conversion, and yields of ethanol.

**The Impact**

An understanding of the cellular mechanisms of IL toxicity can enable rational engineering approaches to design robust microbial strains that efficiently convert IL-processed biomass to biofuels. The chemical genomics approach is a valuable tool that may be used to rapidly tailor existing strains to new deconstruction technologies and biomass-derived hydrolysates.

**Summary**

Chemical genomics was used to identify yeast gene deletions that confer sensitivity or resistance to [EMIM]Cl, an imidazolium-based ionic liquid (IIL), in the yeast *Saccharomyces cerevisiae*. Among the sensitive mutants there was significant enrichment for genes that encode mitochondrial proteins, suggesting that IIL affects mitochondrial function. In support of this, fluorescence microscopy confirmed perturbation in mitochondrial morphology and altered mitochondrial membrane polarization in the presence of IIL. The top resistant mutant contained a deletion in *PTK2*, a putative serine/threonine protein kinase, which is thought to activate the plasma-membrane proton efflux pump Pma1p. Conversely, overexpression of Pma1p conferred sensitivity to IIL, suggesting that hydrogen ion efflux may be coupled to influx of the toxic imidazolium cation. A model for IIL toxicity was proposed whereby IILs target the mitochondrial membrane and the degree of toxicity is influenced by hydrogen ion homeostasis and IIL import into the cell. *PTK2Δ*, when engineered into a biofuel-producing *S. cerevisiae* strain, showed improved sugar conversion and biofuel production, in addition to IIL tolerance. This work demonstrates the utility of chemical genomics-guided biodesign for development of superior microbial biocatalysts for biomass conversion.

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**Publications**

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