JBEI Researchers Discover and Characterize a Tropical Rain Forest Soil Bacterium that May Facilitate a "One-pot" Solution for Biofuels Initiatives

CHALLENGE

Ionic liquids (molten liquid versions of salts) have proven quite effective in the dissolving of cellulose preparative to its digestion to the sugar glucose by bacterial enzymes (for a more detailed discussion, see [LINK TO "**Optimizing the Use of Plant Cellulose as a Liquid Transportation Biofuels Precursor:** ***Sandia research paper captures large readership."***

The problem with this solution is that ionic liquids—because of their extremely high osmotic pressure—are quite toxic to cells, including bacteria, and after solubilizing cellulose from plant biomass, the optimal technical course would be to incubate the now more-digestible cellulose with bacteria that would digest it to glucose and then ferment the glucose to ethanol. This so-called "one-pot" solution places everything from raw untreated plant biomass to ionic liquid to bacteria inside a single reaction vessel and emerges with the biofuel ethanol — a one pot start-to-finish process that would be both efficient and economical for the biofuels industry.

RESEARCH

Researchers at DOE's Joint Bioenergy Institute (JBEI)—Including Sandian Blake Simmons—may have recently taken an important step toward that solution by discovering and partially characterizing a tropical rainforest bacteria that shows marked tolerance to survival and growth in ionic liquids. *Enterobacter lignolyticus* illustrates a variety of genetic/ metabolic adaptations allowing it to exhibit this tolerance.

Preliminary work indicates that its lipid bilayer cell membrane differs in composition, with certain fatty acids modified to form cyclopropane rings, this characteristic reducing membrane fluidity and thus the membrane's leakiness to the penetration of ions in the ionic liquid. In terms of membrane proteins, an analysis of cellular RNA indicates that transcription (expression) of genes for porins (such as ion channels for sodium or chloride ions and perhaps aquaporins—water pores) are down-regulated, thereby further decreasing membrane permeability. Finally genes for certain transporter proteins that actively transport molecules such as sugars and amino acids into the cell are upregulated, as are metabolic pathways within the cell that synthesize such substances. This points to an attempt by the bacterium to increase its internal solute concentration in order to adjust the osmotic pressure of its internal cytoplasm to more closely match the extremely high osmotic pressure of the ionic liquid externally, thereby minimizing the osmotic movement of water (that would tend to flow out of the cell, dehydrating it and probably causing its death).

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SIGNIFICANCE

Should the cells of *Enterobacter lignolyticus* exhibit sufficient resistance to osmotic dehydration in ionic liquids and are able to degrade solubilized cellulose to glucose and ferment it to ethanol—or should its metabolic adaptations point to ways to genetically engineer other bacteria— the one-pot solution from biomass to ethanol would be much closer to feasibility. Although preliminary and somewhat nonspecific, the evaluation of this bacterium points to this possibility.

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