Development of a Biological Alcohol Sensor

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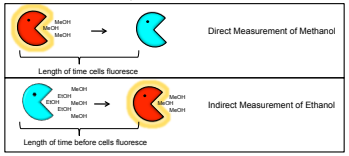
Project Description

The alcohol sensor is a device created from living microorganisms through the techniques of **synthetic biology**. Through genetic alterations the behavior of the microbes may be modified to function as a **living machine**.

The **alcohol sensor** is based on the metabolic activity of the yeast *Pichia pastoris*. Through genetic modification, these cells may indicate the concentration of alcohol present in their environment. Methanol concentration will correspond to the time or intensity of fluorescence. Ethanol concentration may be determined from the length of time it takes for the cells to produce a visible signal after both ethanol and methanol have been introduced to the system. This signal mechanism is created through the fusion of the alcohol oxidase gene (AOXI) promoter and a red fluorescent protein indicator in a vector.



Overview of alcohol sensor device

Applications

The alcohol sensor could be used whenever measurements of ethanol or methanol concentrations are desired. A few examples are listed below.

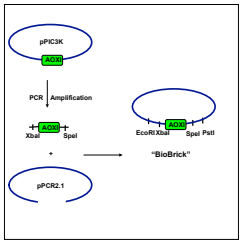
Both ethanol and methanol are added to gasoline to alter oxygen content. The alcohol sensor could be used to determine the concentration of both additives.

The alcohol sensor can also be used in homebrewing. It is well-known that if methanol is not effectively distilled from drinking alcohol it can cause blindness. The alcohol sensor could be an inexpensive test for the safety of homebrewed alcohol.

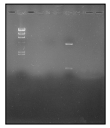
Methanol has numerous industrial applications in synthesis of precursors for synthesis and alternative fuels. Biological and abiological production of methanol is of industrial interest. The alcohol sensor could be used to monitor methanol concentration.

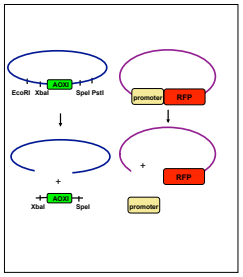
Method and Results

The alcohol sensor is still in the construction phase. Current obstacles include problems with inserting our new BioBrick into the gfp reporter construct. The BioBrick has been created and submitted to the registry as part BBa_I764001 - AOX1 promoter from Pichia pastoris.

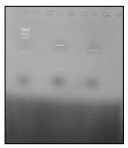


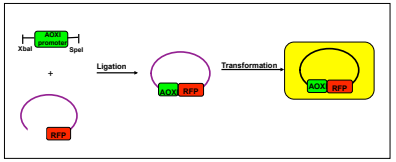
(left)Construction of the biological breathalyzer BioBrick. The AOXI promoter is cut from the pPIC3K vector, flanked with the standardized restriction sites and inserted into a pPcr2.1 vector. (below) Gel electrophoresis after restriction enzyme digest





(left) The AOXI promoter is cut from the pPcr2.1 vector and the promoter is removed from the red fluorescent protein vector obtained from the Parts Registry. (below) Gel electrophoresis after restriction enzyme digest.





The completed alcohol sensor system. The AOXI promoter is fused with the red fluorescent protein gene and transformed into competent cells.

Modeling

Modeling will be necessary to correlate concentration of alcohol and time and intensity. When the device is used as a methanol sensor, the time and intensity of fluorescence will be dependent on the amount of methanol present. When the device is used as an ethanol sensor, the concentration will be dependent on the amount of time before fluorescence occurs. This time will correspond to the consumption of ethanol and the lag time before methanol induces gene expression. Panel B of the figure below from Inan et al illustrates the diauxic growth of *Pichia pastoris* when both ethanol and methanol are present.

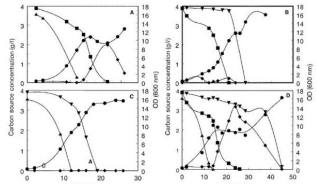


FIG. 1. Growth and Carbon utilization of *P. pastoris*. (A) ethanol-glycerol; (B) ethanol-methanol; (C) methanol-glycerol; (D) ethanol-methanol-glycerol mixtures. Closed circle, OD; closed hexergs, acetate; closed triangle down, methanol; closed triangle up, glycerol; closed square, ethanol.

Obstacles

Self-ligation of the RFP vector has been a problem. The team plans to improve ligation techniques through use of controls and purifications.

The indirect measurement of ethanol concentration presents a complication. First, methanol has to be present for ethanol concentration to be measured. Also, the lag time before the red fluorescence can be seen may be a problem for modeling.

Another potential obstacle is expression of red fluorescence in *E. coli* cells in response to ethanol and methanol. *E. coli* likely lacks the regulatory proteins for proper control of AOX1 expression. The team anticipates eventually needing to use yeast as the chassis rather than *E. coli* to correct this problem.

Attracting members from more disciplines such as material science, electrical and computer engineering, etc.

Discussion

Construction of the alcohol sensor is in progress. Modifications to the design that will correct potential problems such as appropriate expression of red fluorescence are being researched.

DNA sequencing has been performed to confirm that the correct BioBrick has been created. The final BIoBrick fused to rfp will later be transformed into *P. pastoris*. Further testing and mathematical modelling will be conducted to investigate the behavior and response of *P. pastoris* to ethanol.

Though incomplete, the alcohol sensor holds promise. Through our research at Missouri S&T as well as our participation at the iGEM jamboree at MIT, we have found that engineering microorganisms to perform as machines is a challenging but rewarding experience and we learn about team work and develop valuable job skills.

The 2007 Missouri Miners iGEM team. Photo courtesy of B.A. Rupert.

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3. **International Genetically Engineered Machines Competition (iGEM).** http://parts.mit.edu/igem07/index.php/Main_Page.