FOOD WARDEN
It’s rotten... and you know it!

Spoiled-meat detection system

Every year, one third of global food production (~1.3 billion tons of food) is thrown away, partially due to the “best before” dating system. iGEM Groningen 2012 seeks to provide an alternative method of assessing edibility: the Food Warden. It uses an engineered strain of Bacillus subtilis to detect and report volatiles in spoiling meat. Bacillus subtilis was chosen because it can form spores, which can be packaged, stored, and transported.

Volatiles identification

Compounds present in spoiled meat were studied using GC-MS with three organic solvents: water, a range of polarities (methanol, dichloromethane, and hexane). Spoiled meat, fresh meat, and pure solvent were measured and compared (see adjacent spectrum). We found several surprising compounds present in spoiled meat only, like 2,3-dimethylbutane and 2,4-dimethylhexane.

Sensor

Using a microarray approach we discovered that B. subtilis responds to volatiles from rotting meat. We compared the transcriptomes of B. subtilis subjected to volatiles from fresh and spoiled meat using a self-designed setup in which the volatiles flowed through the bacterial culture. The analysis indicated two highly upregulated operons: snoA-sboA-sboX-aib and napC-fnr. Promoters of upregulated operons were used for our detection system: PsoBA and Pmnr.

Results

- We successfully engineered Bacillus subtilis to express amiliCP and amilGFP in response to the volatiles from rotten meat under the regulation of PsoBA (BBa_K818600 & BBa_K818400)
- The characterization data of PsoBA-amiliCP (BBa_K818600) showed a significant difference in the amount of amiliCP (yellow) expressed by our construct under different circumstances (without meat, subjected to volatiles from fresh meat, and subjected to volatiles from spoiled meat).
- Finally, we successfully engineered a packaging system for our Bacillus strain, supporting its growth and ability to produce pigments in response to the volatiles from rotten meat. The picture on the right shows amiliCP (BBa_K592009) produced by our device (BBa_K818400) under the regulation of PsoBA in response to the volatiles from the rotten meat.

Sticker

We designed and tested the Sticker, a capsule which contains bacteria and nutrients, and permits volatiles influx. The precise amount of these nutrients were determined by modeling bacterial growth using consumption rates from literature, and a thermodynamically-constrained stoichiometric model of Bacillus subtilis. The material for the outer layer was selected for safety, strength, ease of construction and use, and pore size. We use TPX2 as it is food-grade (FDA and EU approved), heat-sealable, and has pores between 10-15 mm (10x smaller than B. subtilis spores). In addition, modeling provided a means of calculating the minimum surface area of TPX2 required to permit the maximum oxygen uptake rate.

Construct

Our construct idea: production of pigment regulated by a specific promoter. The volatiles from the rotten meat induce the corresponding promoter to become active, thus allowing the transcription of downstream pigment genes. We used our own B. subtilis backbone (BBa_K818000). It integrates into the Bacillus subtilis chromosome and has an E. coli origin of replication.

Pigments

The use of pigment-based reporter in our device to make it super user friendly. The pigments lycopene (BBa_K274100), amiliCP (BBa_K592009), and amilGFP (BBa_K592010) are easily visible to the naked eye once activated by the volatiles. Pigment-related BioBricks have been developed for use in E. coli, but not in Bacillus subtilis. We successfully expressed them in our bacterium of choice.

Market research

The Food Warden works! The bacteria detect rotten meat while inside the sticker. It’s a functional prototype that could be developed to a product on the market. We conducted a survey for which we received 167 responses, of which 82.5% claimed they would like to see a product like this on the market, and 67.7% of them are willing to pay extra to have it included. We estimated the production costs of a single sticker to be around 3.5 euro cents.