What is UCP?
The hot yeast project:
Can we build a microorganism that regulates its own temperature?

[ + UCP = ? ]
Estimation of temperature increase:

Glucose → H+ → ATP

Glucose → H+ → Heat

ΔT = 30K

This is assuming:

- No heat losses to the environment.
- Glucose is only metabolized through respiratory transport.
- No protons through ATPase.
The hot yeast project:

\[ + \quad \text{UCP} = \quad \text{Hot yeast} \]
Applications

Having a living cell which maintains its temperature between certain levels without the need of introducing external heat to the system. This system would allow the reduction of electricity costs in an specific reaction.
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Implementing this system in some plants species. If a plant could control its temperature it would be able to grow in colder climates or to survive frosts.
Objectives

1.- Construction of a new system consisting of Liquid Culture Calorimeter (LCC): a calorimeter with a thermocouple inside allowing microbial growth. This is an isolated system that is able to maintain temperature around 28 °C and record it during the experiment with a precision of ± 0.1 °C.
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2.- Demonstration that thermogenin-expressing yeast strains can heat their own broth medium.
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2.- Demonstration that thermogenin-expressing yeast strains can heat their own broth medium.

3.- Implementation of a regulatory system through genetic engineering and synthetic biology of yeast strains allowing them to maintain the temperature of the medium around the wanted interval.
LCC Construction
LCC Characterization

Conditions:

- 100 ml in a 1L capacity LCC

- Initial temperature similar as further experiments
Strains/Plasmids

UCP+
UCP Gly 175Δ
UCP Gly 76 Δ
UCP-

Gal7 promoter +

Plasmid pYEDPr
The delayed growth for mutants UCP Gly 175Δ, UCP Gly 76Δ confirm that active thermogenin is being produced.
Preliminary Experiments

Conditions:
- 250 rpm and slightly tilt
- Initial OD: 0.2
- Induction at the beginning with 1% galactose
  + Extra galactose shot after 9 hours and left 50 hours
UCP expression

Conditions:
- 275 rpm and slightly tilt
- Initial OD: 0.6
- Induction at the beginning with 1% galactose
Modeling

\[ N = N_r (1 - \frac{N}{K}) \]

\[ G = -\beta_1 N \]

\[ H = \alpha \left( \frac{G}{K_1} \right)^n - \beta_2 H + \gamma \]

\[ T = -k (T - T_a) + \xi H \]
Modeling

\[ N = Nr \left(1 - \frac{N}{K}\right) \]

\[ G = -\beta_1 N \]

\[
H = \alpha \frac{\left(\frac{G}{K_1}\right)^n}{1 + \left(\frac{G}{K_1}\right)^n} - \beta_2 H + \gamma
\]

\[ T = -k (T - T_a) + \xi H \]

Equation for growth simulation

Equation for galactose consumption

Equation for thermogenin expression

Equation for temperature evolution

Promoter related

Constant for all strains
Modeling

- Equation for growth simulation
  \[ N = N_r \left( 1 - \frac{N}{K} \right) \]
- Equation for galactose consumption
  \[ G = -\beta_1 N \]
- Equation for thermogenin expression
  \[ H = \alpha \left( \frac{G}{K_1} \right)^n \left( 1 + \left( \frac{G}{K_1} \right)^n \right) - \beta_2 H + \gamma \]
- Equation for temperature evolution
  \[ T = -k (T - T_a) + \xi H \]

Calculated for each strain
Modeling

Growth curve

\[ N = N_r(1 - \frac{N}{K}) \]
\[ G = -\beta_1 N \]

Equation for growth simulation

Equation for galactose consumption

\[ H = \alpha \left(\frac{G}{K_1}\right)^n \left[ \left(\frac{G}{K_1}\right)^n - \beta_2 H + \gamma \right] \]
\[ T = -k(T - T_a) + \xi H \]

Equation for thermogenin expression

Equation for temperature evolution

LCC characterization curve
- UCP1 has a long half life.
- Galactose is consumed in the first two-three hours.
- Caloric effect is slightly higher for 175 Δ than for 76 Δ, as shown by ξ.
- In UCP-, value for ξ was almost 0.

### Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
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<tbody>
<tr>
<td>α</td>
<td>6.9±0.4</td>
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<tr>
<td>K₁</td>
<td>0.15±0.08</td>
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<tr>
<td>β₂</td>
<td>0.034±0.006</td>
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<tr>
<td>γ</td>
<td>0.012±0.003</td>
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<tr>
<td>n</td>
<td>2</td>
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### Gal. Consumption and Heat effect

<table>
<thead>
<tr>
<th>Parameters</th>
<th>175 Δ</th>
<th>76 Δ</th>
<th>UCP-</th>
</tr>
</thead>
<tbody>
<tr>
<td>β₁</td>
<td>0.109±0.007</td>
<td>0.09±0.05</td>
<td>0.90±0.07</td>
</tr>
<tr>
<td>ξ</td>
<td>3.7±0.3</td>
<td>3.14±0.89</td>
<td>0*</td>
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</tbody>
</table>
Modeling the control system: a thermostat for the heating system
Modeling the control system: a thermostat for the heating system

Problems:

- Cold shock/heat shock proteins work best in a range or temperature not entirely suitable for our experiment.

- UCP1 has an long half life. Even if you stop expression, the protein remains present and active for a long period of time.
Conclusions

We have:

Developed a brand new instrument: LCC (Liquid Culture Calorimeter).

Demonstrated, for the first time, that UCP-expressing yeasts are able to produce a significant increase in temperature.

Characterized four new biobricks (a UCP-expressing yeast, a control strain and two mutant strains with enhanced uncoupling activity).

Developed an effective model to characterize the kinetic behavior of thermogenin.
Conclusions

We have:


Further work...

Design of a regulatory system of the thermogenin-associated thermal increase.
Thank-you!

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