TEAM iGEM IISER BHOPAL

E.L.S.A : E.COLI LEARNING SUB - OPTIMAL ACCLIMATIZATION

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Introduction

- The intrinsic characteristics of psychrophilic enzymes, high activity at low temperatures and thermolability, are valuable for different biotechnological applications, ranging from molecular biology to detergency to food and beverage preparation.
- Production of such enzymes in commonly used mesophilic expression systems is hampered by their low stability at the optimal host growth temperatures. Several mesophilic proteins overexpressed in *E. coli* tend to form inclusion bodies due to improper folding which reduces the soluble yield.
- A strategy used is the reduction of the growth temperature, which improves the soluble yield marginally, but reduces the biomass.
- Thus, our plan is to construct a plasmid that confers cold tolerance to the most industrially relevant microorganism, *E. coli* which was obtained by integrating a heterologous chaperone/ chaperonin system.

Methodology & Result

Ligation of pUC18 with Cpn10 and Cpn60
Well 1 - Standard DNA Ladder
Well 2 - pUC18 (2686 bp)
Well 3 - pUC18 fused with Cpn10 and Cpn60 (4636bp)

Ligation of pUC18 plasmid with Cpn60
Well 1 - 1 Kb ladder with standard molecular weight
Well 2 - pUC18 plasmid (2686 bp)
Well 3 - Ligated product of Cpn60 and pUC18 (4336 bp)

Cloned Trigger Factor of *P. haloplanktis* in pUC18
Well 1 - 1 Kb Ladder with standard molecular weight
Well 2 - pUC18 plasmid (2686 bp)
Well 3 - pUC18 fused with Trigger Factor of *P. haloplanktis* (1348 bp + 2686 bp = 4034 bp)

Growth Analysis

Modelling

The growth rate of our system may be modelled as a function of growth temperature and chaperones expressed, as governed by the Ratkowsky's equation:

$$R(T, \text{chap}) = a * [(T - T_{\min}) * (1 - e^{-b * (T - T_{\max})})]^2$$

a, b: Constants unique to the modified bacterial strain (reflects the effect of TF)

Min: temperature below which growth is insignificant

Constant

Temperature at which the culture is grown

Constant

Limiting temperature above which growth is insignificant

For temperatures below that of optimum growth, the value of b tends to infinity. Therefore the second term reduces to 1. Thus, the simplified equation is

$$\sqrt{r} = k (T - T_{\min})$$

Here on, the idea is to obtain growth curves of the systems at different temperatures, to plot the square root of growth rates as a linear function of temperature.

System	Tmin (°C)	Goodness of Fit (R ²)	Standard Deviation
pUC18	9.5	0.7749	0.0095
Cpn60	-12.0	0.8425	0.00502
Cpn10/60	-5.0	0.997	0.000641
TF	8.9	0.999	0.000627

Future directions

- Our primary aim will be the completion of our project, with all three systems in one plasmid. This will allow us to then check for the percentage increase in the soluble fraction of certain industrially relevant enzymes.
- Replace the innate *E. coli* with the Trigger factor from *P. haloplanktis* and integrating the *O. antartica* chaperonins in the same system.
- Development of a cold-tolerant chassis which could serve as a replacement to the currently dominant mesophilic chassis in enzyme production.
- Utilizing the cold tolerance of our construct as a selectable marker to replace the antibiotic resistance cassette from a cloning vector. This would help reduce the usage of antibiotics in research laboratories.

Support / Sponsors

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