

Carbon fixing bacteria

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Overview

The two most pressing challenges facing humanity are the need to feed the growing population with restricted land and water sources and global warming caused due to high atmospheric CO₂ levels. Biological systems for CO₂ conversion to organic molecules using renewable-energy-harvesting techniques present a promising approach to simultaneously treating both problems. The lab of Prof. Ron Milo developed a breakthrough synthetic biology approach and engineered bacteria (*E. coli*) to consume CO₂ as a source for their biomass carbon.

Background and Unmet Need

The two most pressing ecological challenges of our time are reducing CO₂ levels in the atmosphere, which is responsible for extreme weather events and creating enough food for the ever-growing population. In light of the tremendous economic costs of global warming, there is a substantial economic incentive to develop new technologies capable of removing CO₂ from the atmosphere. Importantly, today's food supply chain accounts for over 20% of anthropogenic greenhouse gasses emission¹. Therefore, there is a growing need for developing a natural and sustainable food source. Biological systems for CO₂ conversion to organic molecules present a promising avenue due to their high product specificity and modularity.

The Solution

Prof. Ron Milo and his team were the first ever to engineer *E. coli* bacteria to produce all of their biomass from CO₂ as the sole carbon source². Therefore, this technology can address both problems presented above at once: consuming CO₂ for potentially synthesizing food³.

Technology Essence

Our engineered *E. coli* strain uses the Calvin cycle for carbon fixation and harvests energy and reducing power from the one-carbon molecule formate (HCOO⁻), which can be produced electrochemically.

The generation of a fully autotrophic *E. coli* required deleting central genes involved in carbon metabolism (Pfk A,B and Zwf), and expressing specific enzymes facilitating CO₂ assimilation, namely Rubisco and phosphoribulokinase (Prk) for carbon fixation and formate dehydrogenase (FDH) for harvesting energy and reducing power from formate.

Heterologous expression of non-native enzymatic machinery potentially enables autotrophic growth; however, it does not guarantee that the cell will utilize it as a favorable pathway. To drive the flux toward the desired metabolic pathway, the deletions were implemented by rational design, and evolutionary pressure was used by growing cells

in Xylose limited chemostats under continued starvation conditions in the presence of excess formate and CO₂, to select cells with reduced dependence on external carbon source. Autotrophic growth was achieved following several months of continuous laboratory evolution and confirmed via isotopic labeling.

Figure 1: The experimental workflow begins from the left where the E. coli strain is engineered for xylose catabolism (pfkA, pfkB, Zwf deleted; Rubisco, Prk, CA, FDH overexpressed), but is unable to grow in autotrophic conditions. As the limited amount of xylose is consumed in the chemostat, and with an excess of formate and CO₂, the cells are under a strong selection pressure to use CO₂ as the only carbon source, while using formate oxidation by FDH as the energy source. Evolved clones with a fully autotrophic phenotype were then produced (right), as they achieved a fitness advantage over xylose dependent strains and become the dominant population in the chemostat.

Applications and Advantages

- First ever central biotechnological heterotrophic organism switched to become autotrophic.
- A potential modular platform for biotechnological production of various chemicals with net negative CO₂ emissions, by integration of existing synthetic metabolic pathways into a synthetic autotrophic strain.
- Assimilation of atmospheric CO₂ with potential of cheap and facile production of high value products.
- An E. coli line that can be further genetically engineered easily using standard tools.
- Unique research tool to improve enzymes related to the CBB cycle to help in the production of more efficient plant/crop strains.

Development Status

The Milo team has performed the experimental work of generating novel strains of carbon fixing E. coli including characterization both in terms of carbon economy and genomic sequencing to determine the mutations leading to E.coli autotrophy.

References

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