^{13}C Fingerprinting to Track the Flow of Carbon in Metabolic Pathways

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Abstract

Understanding the metabolic pathways by which carbon substrates are assimilated is an important quest in metabolic engineering. The first experiment was performed to analyze the assimilation paths for labeled and unlabeled glucose in E. coli strains engineered for ethanol biosynthesis. Results indicated production of labeled ethanol from the labeled substrate. The second experiment was performed to analyze the E. coli strains cocultured, where one strain was specialized for glucose assimilation while another one for xylose. Both the strains were grown in different compartments of the same reactor. The analyzed data indicated that the glucose specialist strain could release CO2 which would then be assimilated in the xylose specialist strain, based on the labeling results.

Things to Know

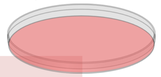
- Metabolic pathways are a series of biochemical reactions utilized by organisms for substrate assimilation
- Different enzymes within the pathway affect substrates differently
- C^{13} is a stable Carbon isotope containing an additional neutron which provides the atom a greater mass making them differentiable during GC-MS
- These atoms are distinguished by using M+n, where n is the number of heavier atoms present within the molecule

	M+0	M+1	M+2	M+3
Alanine	0.48	0.04	0.04	0.44
Glycine	0.51	0.07	0.43	0
Valine	0.61	0.04	0.02	0.02
Leucine (M-15)	0.94	0.05	0.01	0
Isoleucine (M-15)	0.87	0.09	0.04	0
Methionine	0.48	0.11	0.02	0.04

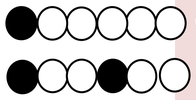
Procedure

Cells cultivated with labeled/ unlabeled carbon

C^{13} ● C^{12} ○



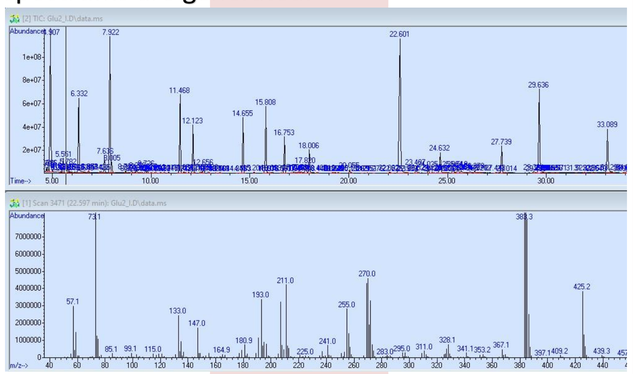
Differentially labeled intermediates form as substrate goes through central pathway



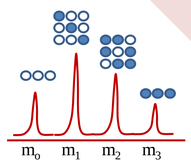
C^{1} labelled glucose

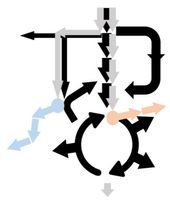
$C^{1,4}$ labelled glucose

GC-MS Analysis is performed and mass spectrums are generated.



MATLAB is used to quantitate data. Analysis of the data allows the pathway taken by the substrate to be determined.





Results

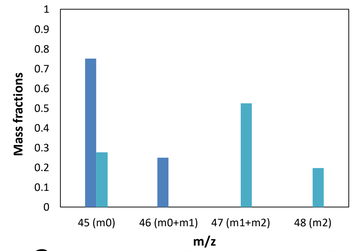
Experiment 1: Ethanol estimation using GC-MS.

- Sample: *E. coli* cells engineered to synthesize ethanol
- Extractant: n-hexane
- Characteristic ethanol m/z: 45
- Ions to be compared: 45 (m0), 46 (m0/m1), 47 (m1/m2), 48 (m2)

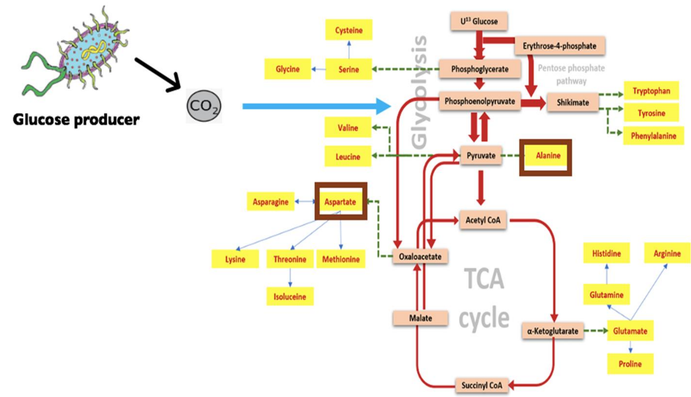
Cell cultures under consideration:

A. E.coli cells grown with ^{12}C-glucose

E. E.coli cells grown with ^{13}U-glucose



Experiment 2: Studying Engineered *E coli* coculture for carbon dioxide assimilation and carbon labelling in central metabolic pathway.



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