Genome-scale Metabolic Reconstruction of the Sf9 Cell

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Abstract

Sf9 cells can produce recombinant proteins such as spider silk using baculoviruses. The genome of the Sf9 cell is currently being sequenced, and once completed, the genome will be annotated. The metabolic genes identified in the Sf9 genome will be used to create a genome-scale metabolic reconstruction of the Sf9 cell, which will be used to optimize bioproduction of spider silk.

Introduction

Figure 1. *Spodoptera frugiperda*
http://commons.wikimedia.org/wiki/File:Spodoptera_frugiperda_worm.jpg

- Sf9 cells are developed from the ovarian tissue of the Fall Army worm, *Spodoptera frugiperda* (Fig. 1)

- These cells are commonly used for recombinant protein production using baculoviruses
- Spider silk is a protein of interest for production in Sf9 cells due to its strength and flexibility (Fig. 2)

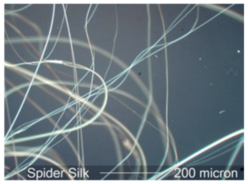

Figure 2. Spider Silk
http://www.microlabgallery.com/gallery/SpiderSilk.aspx

Objectives

- Sequence whole genome of Sf9 cells
- Annotate Sf9 genome to identify metabolic genes
- Create metabolic reconstruction of Sf9 cells using genomic data
- Use metabolic reconstruction to optimize production of recombinant spider silk
- Collect experimental data to validate and refine the reconstruction

Materials and Methods

Whole Genome Sequencing and Annotation

- Sequencing of the DNA of Sf9 cells is currently being performed using 454 Genome Sequencing and the Illumina System
- Total genome size is unknown, but is estimated to be above 3,200 Mb

Reconstruction

- Created using a database of metabolites and reactions (Fig. 3)

Metabolites

Name	Description	Neutral Formula	Charged Formula	Charge
acoa[c]	acetyl-coenz	C23H38N7O17P3	C23H34N7O17P3	-4
adp[c]	adenosine d	C10H15N5O10P2	C10H12N5O10P2	-3
akg[c]	a-ketoglutar	C5H6O5	C5H4O5	-2
akg[e]	a-ketoglutar	C5H6O5	C5H4O5	-2
ala[c]	alanine	C3H7NO2	C3H7NO2	0
ala[e]	alanine	C3H7NO2	C3H7NO2	0
amm[c]	ammonia	NH3	NH4	1
amm[e]	ammonia	NH3	NH4	1
arg[c]	arginine	C6H14N4O2	C6H15N4O2	1

Reactions

Name	Description	Formula	Cellular Sut	R
GAP	Glyceraldeł	gap[c] + nad[c] + adp[c] + pi[	Glycolysis a	
PYK	ATP:pyruva	adp[c] + h[c] + pep[c] -> pyr[	Glycolysis a	
PPP	pentose-ph	3 g6p[c] + 6 nadp[c] + 3 h2o[	Glycolysis a	
LDH	Lactate:NAI	pyr[c] + nadh[c] + h[c] -> lac[	Pyruvate N	
ALAAT	Alanine:2-o	pyr[c] + glu[c] -> ala[c] + akg[	Pyruvate N	
PDH	pyruvate:N.	pyr[c] + coa[c] + nad[c] -> co2	Pyruvate N	
ME	malate:NAI	mal[c] + nad[c] -> pyr[c] + co2	Pyruvate N	
CS	acetyl-CoA:	acoa[c] + oaa[c] + h2o[c] -> c	TCA Cycle	
CITDC	Isocitrate:N	cit[c] + nad[c] -> co2[c] + akg	TCA Cycle	
AKGDH	2-Oxogluta	akg[c] + coa[c] + nad[c] -> co2	TCA Cycle	
SUCOAS	succinyl co	sucoa[c] + adp[c] + pi[c] -> su	TCA Cycle	
SUCDH	Succinate:(	suc[c] + fad[c] -> fum[c] + fad	TCA Cycle	

Figure 3. A partial list of metabolites and reactions listed in the databases

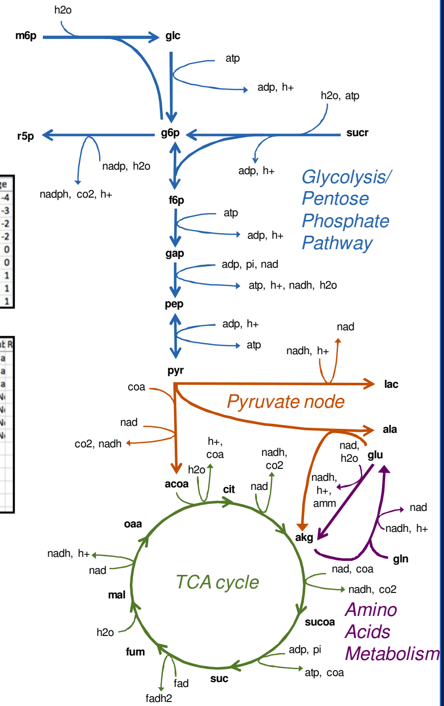

Figure 4. Simplified metabolic core of Sf9 cells. Glycolysis, PPP, Pyruvate node, TCA cycle, and several amino acid metabolism reactions, transport reactions, and a biomass reaction (not shown) are included. Gluconeogenesis does not operate efficiently in insect cells (Bernal).

- Based on core metabolism of Sf9 cells (Fig. 4)
- Methods for bioproduction optimization include:
  - Gene knockouts
  - Gene additions
  - Media optimization

Future Work

- Once sequencing results are obtained, genome annotation will be performed
- The metabolic genes identified in the Sf9 genome will be incorporated into the reconstruction
- Constraints will be set on the upper and lower bounds for the fluxes in the reconstruction, and flux balance analysis will be used to maximize spider silk production
- Sf9 cells, as shown in Fig. 5, will be grown and various parameters (e.g. growth rate, metabolite production and consumption rates) will be measured to refine and validate the metabolic reconstruction

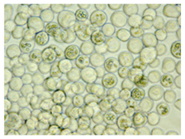

Figure 5. Sf9 cells infected with a baculovirus
http://www2.bioc.cam.ac.uk/baculovirus/

References

Bernal, Vicente, Nuno Carinhas, et al. (2009). “Cell Density Effect in the Baculovirus-Insect Cells System: A Quantitative Analysis of Energetic Metabolism.” Biotechnology and Bioengineering 104: 162-180

Thiele, I. and B. O. Palsson (2010). "A protocol for generating a high-quality genome-scale metabolic reconstruction." Nature protocols 5(1): 93-121.

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