

ADDITIONAL FILE 1: Metabolic reactions of the *Spodoptera frugiperda* cell line (Sf9) metabolism.

For a description of the following, see Bernal et al. (2009) and Carinhas et al. (2010).

The following measured exchange fluxes were considered as constraints for MFA calculations.

Transporters/passive diffusion

$$\text{Glc}_e = \text{Glc}$$

$$\text{Malt}_e = \text{Malt}$$

$$\text{Sucr}_e = \text{Sucr}$$

$$\text{Lac} = \text{Lac}_e$$

$$\text{Amm} = \text{Amm}_e$$

$$\text{Pyr}_e = \text{Pyr}$$

$$\alpha\text{KG}_e = \alpha\text{KG}$$

Amino acids Transport

$$\text{Glu}_e + 1 \text{ ATP} = \text{Glu} + 1 \text{ ADP}$$

$$\text{Asp}_e + 1 \text{ ATP} = \text{Asp} + 1 \text{ ADP}$$

$$\text{Gln}_e + 0.33 \text{ ATP} = \text{Gln} + 0.33 \text{ ADP}$$

$$\text{Ser}_e + 0.33 \text{ ATP} = \text{Ser} + 0.33 \text{ ADP}$$

$$\text{Gly}_e + 0.33 \text{ ATP} = \text{Gly} + 0.33 \text{ ADP}$$

$$\text{Thr}_e + 0.33 \text{ ATP} = \text{Thr} + 0.33 \text{ ADP}$$

$$\text{Met}_e + 0.33 \text{ ATP} = \text{Met} + 0.33 \text{ ADP}$$

$$\text{Pro}_e + 0.33 \text{ ATP} = \text{Pro} + 0.33 \text{ ADP}$$

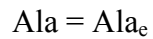
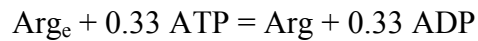
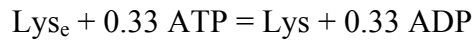
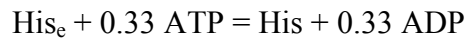
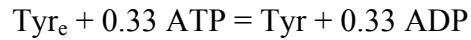
$$\text{Asn}_e + 0.33 \text{ ATP} = \text{Asn} + 0.33 \text{ ADP}$$

$$\text{Leu}_e + 0.33 \text{ ATP} = \text{Leu} + 0.33 \text{ ADP}$$

$$\text{Ile}_e + 0.33 \text{ ATP} = \text{Ile} + 0.33 \text{ ADP}$$

$$\text{Val}_e + 0.33 \text{ ATP} = \text{Val} + 0.33 \text{ ADP}$$

$$\text{Phe}_e + 0.33 \text{ ATP} = \text{Phe} + 0.33 \text{ ADP}$$

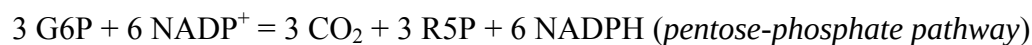
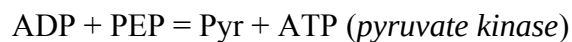
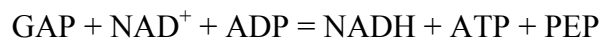
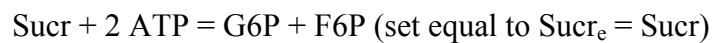
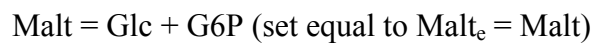
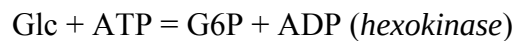


Biomass formation

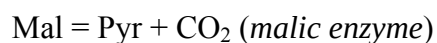
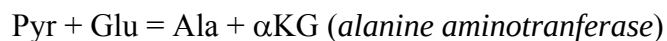
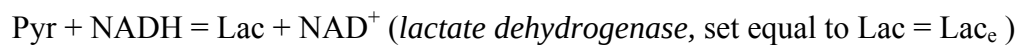
μ

Metabolic reactions of *S. frugiperda*'s metabolism estimated by MFA and used for PLS modelling.

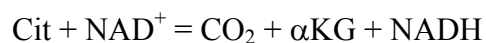
Glycolysis and pentose-phosphate pathway (PPP)



Pyruvate node



TCA cycle



$\alpha\text{KG} + \text{NAD}^+ = \text{CO}_2 + \text{SuCoA} + \text{NADH}$ (*αKG dehydrogenase*)

$\text{SuCoA} + \text{ADP} = \text{Suc} + \text{ATP}$ (*succinyl coenzyme A synthetase*)

$\text{Suc} + \text{FAD} = \text{Fum} + \text{FADH}_2$ (*succinate dehydrogenase*)

$\text{Fum} = \text{Mal}$ (*fumarase*)

$\text{Mal} + \text{NAD}^+ = \text{OAA} + \text{NADH}$ (*malate dehydrogenase*)

Amino acids metabolism

$\alpha\text{KG} + \text{Gln} + \text{NADH} = 2 \text{Glu} + \text{NAD}^+$ (*glutamate synthase*)

$\text{Glu} + \text{NAD}^+ = \alpha\text{KG} + \text{Amm} + \text{NADH}$ (*glutamate dehydrogenase*)

$2 \text{Gly} + \text{NAD}^+ = \text{CO}_2 + \text{Amm} + \text{Ser} + \text{NADH}$

$\text{Ser} = \text{Pyr} + \text{Amm}$

$2 \alpha\text{KG} + \text{Lys} + 4 \text{NAD}^+ + \text{NADPH} + \text{FAD} = 2 \text{CO}_2 + 2 \text{Glu} + 2 \text{ACoA} + 4 \text{NADH} + \text{NADP} + \text{FADH}_2$

$\alpha\text{KG} + \text{Leu} + \text{NAD}^+ + \text{ATP} + \text{FAD} = \text{CO}_2 + \text{Glu} + 2 \text{ACoA} + \text{NADH} + \text{ADP} + \text{FADH}_2 + \text{mevalonate}$

$\alpha\text{KG} + \text{Ile} + 2 \text{NAD}^+ + \text{ATP} + \text{FAD} = \text{CO}_2 + \text{Glu} + \text{ACoA} + \text{SuCoA} + 2 \text{NADH} + \text{ADP} + \text{FADH}_2$

$\alpha\text{KG} + \text{Arg} + \text{NAD}^+ = 2 \text{Glu} + \text{NADH} + \text{urea}$

$\text{Glu} + \text{OAA} = \alpha\text{KG} + \text{Asp}$ (*aspartate aminotransferase*)

$\text{Thr} + \text{NAD}^+ = \text{ACoA} + \text{Gly} + \text{NADH}$

$\alpha\text{KG} + \text{Val} + 3 \text{NAD}^+ + \text{ATP} + \text{FAD} = \text{CO}_2 + \text{Glu} + \text{SuCoA} + 3 \text{NADH} + \text{ADP} + \text{FADH}_2$

$\text{Ser} + \text{Met} + \text{NAD}^+ + \text{ATP} = \text{SuCoA} + \text{Amm} + \text{Cys} + \text{NADH} + 2 \text{ADP}$

$\text{Phe} + \text{NADH} + \text{O}_2 = \text{Tyr} + \text{NAD}^+$

$\alpha\text{KG} + \text{Tyr} + \text{O}_2 = \text{CO}_2 + \text{Glu} + \text{ACoA} + \text{Fum}$

$\text{His} = \text{Glu} + \text{Amm}$

$\text{Asn} = \text{Amm} + \text{Asp}$

$\text{Pro} + \text{NADP}^+ + 0.5 \text{O}_2 = \text{Glu} + \text{NADPH}$

Respiration fluxes

$\text{NADH} + 2.5 \text{ADP} + 0.5 \text{O}_2 = \text{NAD}^+ + 2.5 \text{ATP}$

$1.5 \text{ADP} + \text{FADH}_2 + 0.5 \text{O}_2 = 2.5 \text{ATP} + \text{FAD}$

ATP = Energy

$O_{2,e} = O_2$

$CO_2 = CO_{2,e}$

Transhydrogenase

$NAD^+ + NADPH = NADH + NADP^+$

Biomass synthesis

$0.5 CO_2 + R5P + 1.9 Gln + 1.3 Ser + 1.3 Asp + 0.7 NAD^+ + NADPH + 9 ATP = 1.9 Glu + 0.8 Fum + 0.8 Gly + DNA + 0.7 NADH + NADP^+ + 9 ADP$

$CO_2 + R5P + 1.9 Gln + Ser + 1.3 Asp + NAD^+ + ATP = Glu + 0.8 Fum + 0.5 Gly + RNA + NADH + ADP$

$0.417 Ala + 0.251 Glu + 0.315 Gln + 0.432 Gly + 0.348 Ser + 0.344 Lys + 0.410 Leu + 0.213 Ile + 0.301 Arg + 0.238 Asp + 0.290 Thr + 0.305 Val + 0.109 Met + 0.162 Phe + 0.131 Tyr + 0.109 His + 0.266 Pro + 0.223 Asn + 0.142 Cys + 10.014 ATP = 1 Protein + 10.014 ADP$

$9 AcoA + 7 NADPH + 26 ATP + 10 NADH + 1 O_2 = 1 FA + 7 NADP^+ + 26 ADP + 10 NAD^+$

$1000 Protein + 111 FA + 89.5 DNA + 224.7 RNA = Biomass$

(The balances of ADP, urea, mevalonate and “Energy” could not be closed, as these metabolites intervene in biochemical processes not considered in the metabolic network.)

References

Bernal V, Carinhas N, Yokomizo AY, Carrondo MJT, Alves PM: **Cell density effect in the Baculovirus-Insect Cells system: a quantitative analysis of energetic metabolism.** *Biotechnol Bioeng* 2009, **104**:162-180.

Carinhas N, Bernal V, Monteiro F, Carrondo MJT, Oliveira R, Alves PM: **Improving baculovirus production at high cell density through manipulation of energy metabolism.** *Metab Eng* 2010, **12**:39-52.