Flux analysis of central metabolic pathways in the Fe (III)-reducing organism *Geobacter metallireducens* via ^{13}C isotopic labeling

Romy Chakraborty, Yinjie J. Tang, Héctor García Martín, Jeannie Chu, Terry Hazen, Jay D. Keasling

Geobacter metallireducens is one of only a few iron-reducing microorganisms that have been sequenced. While the genome sequence and proteome are important for understanding a microorganism, they are not necessarily accurate representations of cell physiology and metabolism. Labeling information combined with metabolite balancing and growth kinetics gave an actual *in vivo* picture of *G. metallireducens* metabolism under iron reducing conditions. We analyzed the carbon fluxes in central metabolism of strain GS-15 using ^{13}C isotopomer modeling. Acetate labeled in the 1^{st} or 2^{nd} position was a carbon source, and Fe-NTA was the sole terminal electron acceptor. The resulting isotope labeling pattern of amino acids allowed an accurate determination of the *in vivo* global metabolic reaction rates (fluxes) through central pathways using an isotopomer computation model. The model showed the acetate uptake rate is 21 mmol/gdw/h at the exponential growth phase and over 90% acetate is completely oxidized via TCA cycle. Further, the isotopomer model indicated that the pyruvate carboxylase and phosphoenolpyruvate carboxykinase enzyme are present under these conditions but that the glyoxylate shunt and malic enzyme are absent. Glycolysis and the pentose phosphate pathway were mainly employed for biosynthesis and accounted for less than 3% of total carbon consumption. The model also indicated that the rate-limiting step responsible for slow growth of *Geobacter metallireducens* on Fe-NTA and acetate is acetyl-CoA transferase. This is based on the surprisingly high reversibility in the reaction between oxoglutarate and succinate (enzyme name). These findings enable a better understanding of the relationship between genome annotation and actual metabolic pathways in *G. metallireducens,* and provide complementary flux information to the recent *in silico* model predictions, to further extend our understanding of anaerobic carbon metabolism in this organism.