Inverse Metabolic Engineering of 
Synechocystis PCC 6803 for Improved 
Growth Rate and Poly-3-hydroxybutyrate 
Production

Keith E. Tyo, Greg Stephanopoulos

Massachusetts Institute of Technology

Abstract – Synechocystis PCC 6803 is a 
photosynthetic bacterium that has the potential to 
make bioproducts from carbon dioxide and light. 
Biochemical production from photosynthetic 
organisms is attractive because it replaces the 
typical bioprocessing steps of crop growth, milling, 
and fermentation, with a one-step photosynthetic 
process. However, low yields and slow growth 
rates limit the economic potential of such 
endeavors. Rational metabolic engineering 
methods are hindered by limited cellular 
knowledge and inadequate models of Synechocystis. 
Instead, inverse metabolic engineering, a scheme 
based on combinatorial gene searches which does 
not require detailed cellular models, but can exploit 
sequence data and existing molecular biological 
techniques, was used to find genes that (1) improve 
the production of the biopolymer poly-3-
hydroxybutyrate (PHB) and (2) increase the 
growth rate. A fluorescence activated cell sorting 
assay was developed to screen for high PHB 
producing clones. Separately, serial sub-culturing 
was used to select clones that improve growth rate. 
Novel gene knock-outs were identified that increase 
PHB production and others that increase the 
specific growth rate. These improvements make 
this system more attractive for industrial use and 
demonstrate the power of inverse metabolic 
ingineering to identify novel phenotype-associated 
genes in poorly understood systems.