

Microarray analysis of TG1/pBS(Kan)Synhox vs. TG1/pBS(Kan)

Objectives

To investigate whether:

1. there is a difference in metabolic activity upon expressing Synhox by using *E. coli* TG1/pBS(Kan)Synhox and TG1/pBS(Kan) when both strains produce hydrogen [*E. coli* TG1/pBS(Kan)Synhox produces 3-fold more hydrogen than TG1/pBS(Kan)]
2. cloning of the cyanobacterium *hoxEFUYH* merely increases hydrogen production by up-regulating the native *E. coli* hydrogenase system

Conclusions

1. Gene expression for the *hya*, *hyb*, *hyc*, and *hyp* operons was not altered significantly between TG1/pBS(Kan)Synhox and TG1/pBS(Kan); namely, functional HoxEFUYH is necessary for enhanced hydrogen production in *E. coli*.
2. Using a cut-off ratio of 5.3 (roughly the standard deviation for all the genes), 33 genes were induced and 17 were repressed that are related to other aspects of cell metabolism.

Strains

1. *E. coli* TG1/pBS(Kan)Synhox
2. *E. coli* TG1/pBS(Kan)

Procedures

1. Single colonies from one day-old streaked cultures were used to inoculate complex medium (without glucose but with fructose) and allowed to incubate aerobically at 37 °C overnight for the two strains.
2. The aerobic overnight cultures, fresh medium (including 5 mM IPTG), and sealed crimp-top vials (27 mL) were sparged for 5 min with nitrogen, and then 19 mL of fresh medium and the overnight culture (1 mL, sparged) were mixed into sealed crimp-top vials in a glove box.
3. These vials were anaerobically incubated at 37 °C for 6 hour. After 6 hour, these cultures were used for isolation of total DNA. Cell cultures (1.8 mL) were collected into chilled bead beater vials using a syringe.

Table 1. Induced genes for TG1/pBS(Kan)Synhox vs. TG1/pBS(Kan).

| Gene name | b-# | Signal | Detection | Fold change | Base line signal | Base line detection | Gene function |
|-----------------------------------------------------------|-------|---------|-----------|-------------|------------------|---------------------|------------------------------------------------------------------------------|
| Amino acid catabolism genes (tryptophan) | | | | | | | |
| <i>tnaL</i> | b3707 | 13938.7 | P | 24.3 | 549.7 | P | tryptophanase leader peptide |
| <i>tnaA</i> | b3708 | 3354.3 | P | 13.9 | 244.7 | P | subunit of L-cysteine desulfhydrase / tryptophanase |
| Amino acid catabolism genes (threonine catabolism) | | | | | | | |
| <i>tdcB</i> | b3117 | 1611.8 | P | 9.8 | 195.1 | P | subunit of threonine dehydratase (catabolic) |
| <i>tdcC</i> | b3116 | 1591.3 | P | 8.6 | 182.4 | P | TdcC threonine STP transporter |
| <i>tdcD</i> | b3115 | 1278.8 | P | 8.0 | 202.6 | P | propionate kinase / acetate kinase C |
| <i>tdcE</i> | b3114 | 2218.4 | P | 6.5 | 351.3 | P | 2-ketobutyrate formate-lyase / pyruvate formate-lyase 4 |
| Prophage genes and phage related function genes | | | | | | | |
| <i>ydfX</i> | b1568 | 193.2 | P | 34.3 | 6.4 | A | hypothetical protein |
| <i>pspA</i> | b1304 | 4034.6 | P | 9.2 | 589 | P | regulatory protein for the phage shock protein operon |
| <i>pspB</i> | b1305 | 6335 | P | 8.0 | 753.8 | P | stimulates PspC-mediated transcriptional activation of the <i>psp</i> operon |
| <i>pspC</i> | b1306 | 1971.6 | P | 5.7 | 380.6 | P | PspC transcriptional regulator |
| <i>nmpC</i> | b0553 | 1154.1 | P | 6.1 | 208.5 | P | outer membrane porin protein |
| Energy production/transport (electron donor) genes | | | | | | | |
| <i>sdhC</i> | b0721 | 1438.1 | P | 10.6 | 154 | P | succinate dehydrogenase membrane protein |
| <i>sdhD</i> | b0722 | 2005.7 | P | 9.2 | 186.9 | P | succinate dehydrogenase membrane protein |
| Energy metabolism (TCA cycle) genes | | | | | | | |
| <i>gltA</i> | b0720 | 2226.6 | P | 8.0 | 335 | P | subunit of citrate synthase |
| <i>mdh</i> | b3236 | 2383.6 | P | 5.7 | 424.7 | P | subunit of malate dehydrogenase |
| <i>sucC</i> | b0728 | 2869.6 | P | 5.3 | 454.7 | P | succinyl-CoA synthetase, β subunit |
| Phosphotransferase System genes | | | | | | | |
| <i>treB</i> | b4240 | 2321.8 | P | 9.2 | 380.3 | P | subunit of EIITre |

| | | | | | | | |
|-------------------------------------------------|-------|---------|---|-----|--------|---|--------------------------------------------|
| <i>treC</i> | b4239 | 1181.6 | P | 6.1 | 174.2 | M | trehalose-6-phosphate hydrolase |
| <i>gatA</i> | b2094 | 14585.4 | P | 6.1 | 2506.2 | P | subunit of EIIGat |
| <i>gatB</i> | b2093 | 8325.5 | P | 7.5 | 1325.5 | P | subunit of EIIGat |
| Transporter genes | | | | | | | |
| <i>glpT</i> | b2240 | 4895.1 | P | 6.5 | 767.6 | P | glycerol-3-P MFS transporter |
| <i>yhaM</i> | b3108 | 2518.2 | P | 5.3 | 727.3 | P | hypothetical ORF |
| <i>yhaN</i> | b3109 | 5431.5 | P | 8.6 | 605.3 | P | hypothetical ORF |
| <i>yhaO</i> | b3110 | 3790.1 | P | 9.8 | 411 | P | YhaO STP transporter |
| <i>yhaR</i> | b3113 | 1558.9 | P | 5.7 | 277.2 | P | hypothetical ORF |
| Adaptations (temperature extremes) genes | | | | | | | |
| <i>ibpB</i> | b3686 | 7293.4 | P | 6.5 | 1220.5 | P | small heat shock protein IbpB |
| Sulfur metabolism gene | | | | | | | |
| <i>ydeN</i> | b1498 | 1434.2 | P | 6.5 | 214.4 | P | putative sulfatase |
| Outer membrane protein gene | | | | | | | |
| <i>yciD</i> | b1256 | 1787.5 | P | 8.0 | 256.3 | P | subunit of Colicin S4 Transport System |
| tRNA genes | | | | | | | |
| <i>valT</i> | b0744 | 17792 | P | 6.1 | 2784.7 | P | tRNA for Val (one of seven valine tRNAs) |
| <i>valZ</i> | b0746 | 14873.2 | P | 5.7 | 2452.4 | P | tRNA for Val (one of seven valine tRNAs) |
| <i>valX</i> | b2402 | 14196.8 | P | 5.3 | 2416.2 | P | tRNA for Val (one of seven valine tRNAs) |
| <i>glyW</i> | b1911 | 4921 | P | 5.3 | 803.5 | P | tRNA for Gly (one of six glycine tRNAs) |
| <i>glyV</i> | b4163 | 6106.8 | P | 5.3 | 1243.4 | P | tRNA for Gly (one of six glycine tRNAs) |
| lactose related genes | | | | | | | |
| <i>lacY</i> | b0343 | 3409.5 | P | 5.7 | 612.3 | P | LacY lactose MFS transporter |
| <i>lacA</i> | b0342 | 2377.4 | P | 5.7 | 391.7 | P | subunit of galactoside O-acetyltransferase |

Table 2. Repressed genes for TG1/pBS(Kan)Synhox vs. TG1/pBS(Kan)

| Gene name | b-# | Signal | Detection | Fold change | Base line signal | Base line detection | Gene function |
|-----------------------------------------------------------|-------|--------|-----------|-------------|------------------|---------------------|-----------------------------------------------------|
| Two component regulatory system gene | | | | | | | |
| <i>torT</i> | b0994 | 33.5 | A | -3.0 | 134.4 | P | TorT-unknown inducer |
| Electrochemical potential driven transporter genes | | | | | | | |
| <i>yicE</i> | b3654 | 36.4 | A | -5.3 | 248.3 | P | YicE NCS2 transporter |
| <i>yihP</i> | b3877 | 31.1 | A | -8.0 | 236.7 | P | YihP GPH transporter |
| <i>melB</i> | b4120 | 6.5 | A | -36.8 | 329.6 | P | MelB GPH transporter |
| Prophage genes and phage related function genes | | | | | | | |
| <i>yjJT</i> | b2637 | 9.6 | A | -7.5 | 181.6 | P | CP4-57 prophage; predicted protein |
| <i>intB</i> | b4271 | 25.5 | A | -9.2 | 212.8 | P | KpLE2 phage-like element; predicted integrase |
| Transcription related regulation gene | | | | | | | |
| <i>ydeO</i> | b1499 | 9 | A | -10.6 | 118.8 | P | YdeO transcriptional activator |
| ATP proton motive force interconversion gene | | | | | | | |
| <i>atpC</i> | b3731 | 23.7 | A | -12.1 | 724.9 | P | ATP synthase, F1 complex, ε subunit |
| ABC transporter gene | | | | | | | |
| <i>yjFF</i> | b4231 | 6.5 | A | -34.3 | 190.7 | P | subunit of YtfQ/YtfR/YtfS/YtfT/YjfF ABC transporter |
| TCA cycle gene | | | | | | | |
| <i>fumB</i> | b4122 | 19 | A | -14.9 | 397.6 | P | subunit of fumarase B |
| DNA degradation gene | | | | | | | |
| <i>mcrB</i> | b4346 | 8.1 | A | -32.0 | 229.5 | P | MrcB subunit of 5-methylcytosine restriction system |
| Unassigned reversible reaction gene | | | | | | | |
| <i>sgaE</i> | b4198 | 21.1 | A | -18.4 | 295.5 | P | L-ribulose 5-phosphate 4-epimerase |
| Unknown function genes | | | | | | | |

| | | | | | | | |
|-------------|-------|------|---|-------|-------|---|-------------------|
| <i>yghG</i> | b2971 | 18.6 | A | -5.3 | 92.7 | P | unknown function |
| <i>yjhS</i> | b4309 | 12.3 | A | -6.5 | 119.8 | P | conserved protein |
| <i>yjeN</i> | b4157 | 13.8 | A | -8.0 | 163.6 | P | conserved protein |
| <i>yjjJ</i> | b4385 | 15.6 | A | -9.2 | 196.1 | P | conserved protein |
| <i>yigE</i> | b3814 | 15.9 | A | -11.3 | 228.7 | P | conserved protein |

Table 3. Gene expression for *E. coli* native hydrogenase 3 and the related proteins (formate hydrogenase H and maturation protein) upon cloning *hoxEFUYH*.

| Gene name | pBS(Kan) signal | Synhox signal | Fold change | Gene function |
|-------------|-----------------|---------------|-------------|--------------------------------------------------------------------------|
| <i>hycA</i> | 2538.2 | 1820.7 | -1.3 | Transcriptional repression of hyc and hyp operon |
| <i>hycB</i> | 2589.3 | 1658.9 | -1.6 | Probable small subunit of hydrogenase 3 |
| <i>hycC</i> | 1099.5 | 1218.6 | 1.2 | Membrane-spanning protein of hydrogenase 3 |
| <i>hycD</i> | 827.8 | 1193.9 | 1.3 | Membrane-spanning protein of hydrogenase 3 |
| <i>hycE</i> | 827.8 | 1114.5 | 1.3 | Large subunit of hydrogenase 3 |
| <i>hycF</i> | 1844.1 | 2699.4 | 1.4 | Iron-sulfur protein of hydrogenase 3 |
| <i>hycG</i> | 895.7 | 1215.8 | 1.4 | Hydrogenase activity |
| <i>hycH</i> | 894.7 | 1583 | 1.6 | Processing of large subunit (HycE) of hydrogenase 3 |
| <i>hycI</i> | 1128.4 | 1344 | 1.2 | Protease involved in processing C-terminal end of HycE |
| <i>hypA</i> | 2381.9 | 1545.1 | -1.4 | Pleiotrophic effects on 3 hydrogenase isozyme |
| <i>hypB</i> | 3698 | 2130.2 | -1.7 | Guanosine-nucleotide binding protein, functions as nickel-donor for HycE |
| <i>hypC</i> | 2569.6 | 2331.4 | -1.3 | Pleiotrophic effects on 3 hydrogenase isozyme |
| <i>hypD</i> | 2763.7 | 2032.8 | -1.4 | Pleiotrophic effects on 3 hydrogenase isozyme |
| <i>hypE</i> | 1297 | 1176.8 | 1 | Plays structural role in maturation of all 3 hydrogenase |
| <i>hypF</i> | 389.6 | 521.7 | 1 | Transcriptional regulatory protein |
| <i>fhlA</i> | 493.9 | 715.1 | 1.3 | Transcriptional activator for fdhF, hyc and hyp operon |
| <i>fdhF</i> | 2346.7 | 1193.9 | -1.7 | Selenopolypeptide subunit of formate dehydrogenase H |

Table 4. Gene expression for *E. coli* native hydrogenase 1 and hydrogenase 2 upon cloning *hoxEFUYH*.

| Gene name | pBS(Kan) signal | Synhox signal | Fold change | Gene function |
|-------------|-----------------|---------------|-------------|---------------------------------------------------------------------------------------|
| <i>hyaA</i> | 5877.9 | 5768 | 1.1 | Small subunit of hydrogenase 1 |
| <i>hyaB</i> | 3413.7 | 3011.6 | -1.1 | Large subunit of hydrogenase 1 |
| <i>hyaC</i> | 3199.5 | 2781.5 | -1.1 | Probable Ni/Fe-hydrogenase 1 b-type cytochrome subunit |
| <i>hyaD</i> | 2942 | 2740.4 | -1.1 | Processing of HyaA and HyaB |
| <i>hyaE</i> | 1884.8 | 1701.3 | -1.1 | Processing of HyaA and HyaB |
| <i>hyaF</i> | 908.7 | 1071.7 | 1.3 | Nickel incorporation into hydrogenase 1 protein |
| <i>hybA</i> | 1796 | 2617.8 | 1.4 | hydrogenase 2 4Fe-4S ferredoxin-type component |
| <i>hybB</i> | 557.8 | 870.8 | 1.4 | Probable cytochrome Ni/Fe component of hydrogenase 2 |
| <i>hybC</i> | 365.6 | 566.6 | 1.5 | large subunit of hydrogenase 2 |
| <i>hybD</i> | 522.5 | 489.5 | -1.1 | Probable processing element for hydrogenase 2 |
| <i>hybE</i> | 513.2 | 617.8 | 1.3 | Member of <i>hyb</i> operon |
| <i>hybF</i> | 270 | 380.6 | 1.3 | may modulate levels of hydrogenase 2 |
| <i>hybG</i> | 708.6 | 663.7 | 1 | Hydrogenase 2 operon protein: may effect maturation of large subunit of hydrogenase 2 |