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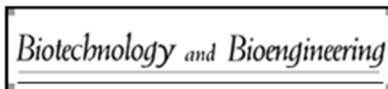
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Improving Product Yields on D-Glucose in Escherichia coli via Knockout of pgi and zwf and Feeding of Supplemental Carbon Sources

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3   **Improving Product Yields on D-Glucose in *Escherichia coli* via Knockout of pgi and zwf**

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9   **and Feeding of Supplemental Carbon Sources**

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17   Running title: Improved product yields on glucose in *E. coli*

18

19   **Abstract**

20   The use of lignocellulosic biomass as a feedstock for microbial fermentation processes presents

21   an opportunity for increasing the yield of bioproducts derived directly from glucose.

22   Lignocellulosic biomass consists of several fermentable sugars, including glucose, xylose, and

23   arabinose. In this study, we investigate the ability of an *E. coli* Δ*pgi* Δ*zwf* mutant to consume

24   alternative carbon sources (xylose, arabinose, and glycerol) for growth while reserving glucose

25   for product formation. Deletion of *pgi* and *zwf* was found to eliminate catabolite repression as

26   well as the ability of *E. coli* to consume glucose for biomass formation. In addition, the yield

27   from glucose of the bioproduct D-glucaric acid was significantly increased in a Δ*pgi* Δ*zwf* strain.

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29   **Keywords**

30   Product yield

31   Strain engineering

32   Biomass

33   D-glucaric acid

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1. Introduction

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36   In recent years, concerns over declining petroleum reserves and climate change due to

37   atmospheric carbon dioxide accumulation have spurred significant interest in using alternative

38   feedstocks for the manufacture of petroleum-derived products. Because it is renewable and

39   abundant, non-food plant (lignocellulosic) biomass represents a promising alternative feedstock

40   to crude oil. Moreover, because plants assimilate carbon dioxide during growth, the use of plant-

41   based feedstocks could potentially slow the accumulation of carbon dioxide in the atmosphere.

42   Recent research has focused heavily on the identification of ideal plant biomass feedstocks

43   (Joyce and Stewart, 2012), the determination of commercially valuable, biomass-derived

44   products (Werpy and Petersen, 2004), and the development of processes for converting plant

45   biomass into these products of interest.

46   One such process that has received heavy attention is microbial fermentation. A significant

47   portion of lignocellulosic biomass consists of fermentable sugars such as glucose, xylose,

48   arabinose, and galactose (Joyce and Stewart, 2012), and many microbes are naturally able to

49   convert these sugars into products of interest such as biochemicals and biopolymers (Lee, 1996).

50   In addition, microbes can be engineered to produce a wide array of non-natural products via

51   recombinant DNA technology (Curran and Alper, 2012). A few products, including D-glucaric

52   acid (Moon et al., 2009) and D-gluconic acid (Rogers et al., 2006), are derived directly from

53   glucose; however, microbial production of these products generally suffers from low yields, as a

54   portion of the glucose feed is utilized to generate cell biomass. For products such as these that

55   do not require further glucose metabolism through the canonical reduction pathways, eliminating

56   the ability to utilize glucose for growth may address this limitation. We thus set out to design an

57   *E. coli* production platform that utilizes an alternative carbon source such as arabinose or xylose

58   for cell growth, reserving glucose solely for product generation to maximize yield.

59   The main pathways for glucose utilization in *E. coli* are depicted in Figure 1. Glucose enters

60   the cell through the phosphotransferase system (PTS) encoded by *ptsG* and *ptsHI-crr* and is

61   phosphorylated to glucose-6-phosphate in the process. Glucose-6-phosphate can then proceed

62   through the Entner-Dudoroff Pathway via *zwf* or through the Embden-Meyerhoff-Parnas

63   Pathway via *pgi*. The carbon in glucose-6-phosphate can also be stored as glycogen via *pgm*.

64   Previous work to engineer *E. coli* for co-utilization of glucose and alternative carbon sources

65   involved deleting the PTS system (Balderas-Hernández et al., 2011; Solomon et al., 2013; Wang

66   et al., 2011). By eliminating the PTS system, catabolite repression can be eliminated, allowing

67   simultaneous uptake of glucose and a secondary carbon source. However, these strategies also

68   eliminate *E. coli*'s primary method of glucose uptake, and the cell must rely on nonspecific

69   transporters to import glucose into the cell. Subsequent phosphorylation of glucose to glucose-6-

70   phosphate via ATP-dependent *glk* is also required for glucose metabolism in PTS-deficient *E.*

71   *coli*. Overexpression of the galactose:H^+ symporter *galP* and upregulation of *glk* has been

72   shown to recover wild-type growth rates in PTS-deficient strains of *E. coli* (Hernández-Montalvo

73   et al., 2003). However, because glycolytic pathways remain intact in this strain, it is likely that

74   product yields on glucose would remain low. In this study, we explore the behavior of an *E. coli*

75   strain which lacks *pgi* and *zwf* and investigate this strain's ability to produce a glucose-derived

76   product when supplemented with L-arabinose and D-xylose, sugars which are readily available

77   from biomass. Glycerol is also explored as a carbon source, as its price has dropped significantly

78   in recent years due to significant increases in biodiesel production (Johnson and Taconi, 2009).

79   Previous work has demonstrated improved yield of glucose-derived products in a Δ*pgi* Δ*zwf*

80 strain supplemented with mannitol (Kogure et al., 2007; Pandey et al., 2013); however, the price

81 of mannitol remains high relative to glycerol and biomass-derived sugars.

Materials and Methods

2.1 *E. coli* strains and plasmids

84 *E. coli* strains, plasmids, and oligonucleotides used in this study are listed in Table I. All

85 molecular biology manipulations were performed according to standard practices (Sambrook and

86 Russell, 2001). *E. coli* DH10B was used for transformation of cloning reactions and propagation

87 of all plasmids.   Strains M2, M2-2, and M3 were constructed by our group previously

88 (Gonçalves et al., 2013; Shiue and Prather, 2014). Strain M2 was generated via knockout of

89 *endA* and *recA* from *E. coli* MG1655. Strain M2-2 was generated via knockout of *gudD and*

90 *uxaC* from strain M2 to prevent consumption of D-glucaric and D-glucuronic acids during D-

91 glucaric acid production experiments. Strain M3 was derived from strain M2 via knockout of *pgi*

92 and served as an intermediate strain; this strain was not further characterized in this work.

93 Deletion of *zwf* from strain M3 was achieved by P1 transduction with Keio collection strain

94 JW1841-1 as the donor (Baba et al., 2006).   The λDE3 lysogen was then integrated site-

95 specifically into this quadruple knockout strain using a λDE3 Lysogenization Kit (Novagen,

96 Darmstadt, Germany), generating strain M4 (MG1655(DE3) Δ*endA* Δ*recA* Δ*pgi* Δ*zwf*.   To

97 prevent *E. coli* from consuming D-glucuronic and D-glucaric acids, both *gudD* and *uxaC* were

98 deleted from the genome. Deletion of *uxaC* was performed with λ-Red mediated recombination

99 (Datsenko and Wanner, 2000) using pKD46recA (Solomon et al., 2013).   PCR primers

100 pKD13_uxaC_fwd and pKD13_uxaC_rev (Table 1) were used to amplify the recombination

101 cassette from pKD13 (Datsenko and Wanner, 2000), and strain M4 harboring pKD46recA was

102 transformed with this PCR product. The *kan* selection cassette was cured from successful

103 deletion mutants using FLP recombinase expressed from pCP20, generating strain M5. Similar to

104 strain M3, strain M5 served as an intermediate strain only and was not further characterized in

105 this work. Finally, strain M6 (MG1655(DE3) Δ*endA* Δ*recA* Δ*pgi* Δ*zwf* Δ*uxaC* Δ*gudD*) was

106 generated using the same λ-Red mediated recombination method described above; in this case,

107 primers pKD13_gudD_fwd and pKD13_gudD_rev were used to amplify the recombination

108 cassette from pKD13. pRSFD-IN and pTrc-Udh were constructed by our group previously

109 (Moon et al., 2009; Yoon et al., 2009). To construct pRSFD-IN-Udh, pRSFD-IN was first

110 digested with XhoI, end-filled with Klenow enzyme, then digested with EcoRI-compatible MfeI.

111 pTrc-Udh was then digested with EcoRI and SmaI, and the Udh-containing fragment was ligated

112 into digested pRSFD-IN to generate pRSFD-IN-Udh.

113 **2.2 Culture conditions**

114 For determination of growth curves, cultures were grown in 250 mL baffled shake flasks

115 containing 50 mL LB medium supplemented with approximately 10 g/L D-glucose, L-arabinose,

116 glycerol, and/or D-xylose as indicated in Figures 2-5. Seed cultures were grown overnight at

117 30°C and inoculated to an optical density at 600 nm (OD_{600}) of 0.005. Cultures were incubated

118 at 30°C, 250 rpm, and 80% relative humidity for 72 hours. To construct a growth curve, cell

119 densities were measured at regular time intervals on a DU800 Spectrophotometer (Beckman

120 Coulter, Pasadena, CA), with more frequent sampling during the exponential growth phase. For

121 analysis of metabolite concentrations, samples were taken daily, centrifuged to remove cell

122 debris, and the supernatants analyzed via high performance liquid chromatography as described

123 in Section 2.3.

124　　　　For glucaric acid production in rich medium, cultures were grown in 250 mL baffled shake

125　　flasks containing 50 mL LB medium supplemented with 10 g/L D-glucose and 10 g/L L-

126　　arabinose, 10 g/L glycerol, or 10 g/L D-xylose. Cultures were induced at inoculation with 0.1

127　　mM β-D-1-thiogalactopyranoside (IPTG). Ampicillin (100 μg/mL) and kanamycin (30 μg/mL)

128　　were added for plasmid maintenance. Seed cultures were grown overnight at 30°C in LB

129　　medium supplemented with 10 g/L D-glucose and 10 g/L L-arabinose, 10 g/L glycerol, or 10 g/L

130　　D-xylose and inoculated to an OD_{600} of 0.005. Cultures were incubated at 30°C, 250 rpm, and

131　　80% relative humidity for 72 hours. Adequate aeration for product formation was ensured with

132　　the use of baffled shake flasks. Samples were taken daily, centrifuged to remove cell debris, and

133　　the supernatants analyzed for metabolite concentrations.

134　　　　　　For minimal medium experiments, a modified MOPS-buffered medium was used containing

135　　10 g/L D-glucose, 6 g/L L-arabinose, 6 g/L NH_4Cl, 0.4 g/L K_2HPO_4, 2 mM $MgSO_4$, 0.1 mM

136　　$CaCl_2$, 40 mM MOPS, 4 mM tricine, 50 mM NaCl, 100 mM Bis-Tris, 134 μM EDTA, 31 μM

137　　$FeCl_3$, 6.2 μM $ZnCl_3$, 0.76 μM $CuCl_2$, 0.42 μM $CoCl_2$, 1.62 μM H_3BO_3, 0.081 μM $MnCl_2$,

138　　carbenicillin (100 μg/mL), and kanamycin (30 μg/ml). Seed cultures were started using a 1:100

139　　dilution from LB and were grown at 30°C for 48 hours in modified MOPS. Working cultures

140　　were inoculated to an OD_{600} of 0.02 and induced 32 hours after inoculation with 0.1 mM IPTG.

141　　Cultures were incubated at 30°C, 250 rpm, and 80% relative humidity for 110 hours. Adequate

142　　aeration for product formation was ensured with the use of baffled shake flasks. Samples were

143　　taken periodically, centrifuged to remove cell debris, and the supernatants analyzed for

144　　metabolite concentrations.

145　　**2.3 Determination of metabolite concentrations**

146   D-glucose, L-arabinose, glycerol, D-xylose, and D-glucaric acid were quantified from

147   culture supernatants using high performance liquid chromatography (HPLC) on an Agilent

148   Series 1100 or Series 1200 instrument equipped with an Aminex HPX-87H column (300 mm by

149   7.8 mm; Bio-Rad Laboratories, Hercules, CA).   Sulfuric acid (5 mM) was used as the mobile

150   phase at 35°C and a flow rate of 0.6 mL/min in isocratic mode. Compounds were detected and

151   quantified from 10 μL sample injections using refractive index and diode array detectors.

152   Reported metabolite concentrations are the average of triplicate samples, and error bars represent

153   one standard deviation above and below the mean value.

3. Results

155   In *E. coli*, glucose is imported into the cell and phosphorylated to glucose 6-phosphate (G6P)

156   by the phosphotransferase system (PTS). Glucose metabolism then proceeds through two routes

157   (Figure 1): the Embden-Meyerhoff-Parnas Pathway via phosphoglucose isomerase (*pgi*) or the

158   Entner-Dudoroff Pathway via glucose 6-phosphate dehydrogenase (*zwf*).   A third route

159   interconverts glucose 6-phosphate and glucose 1-phosphate via phosphoglucomutase (*pgm*) for

160   glycogen storage and accumulation, though flux through this node is typically extremely low

161   (Chassagnole et al., 2002). To eliminate native consumption of glucose, both *pgi* and *zwf* were

162   deleted from an MG1655-derived strain. Growth on M9 minimal medium supplemented with

163   various carbon sources confirmed that the Δ*pgi* Δ*zwf* mutant does not grow on glucose but

164   retains the ability to utilize other carbon sources (Supplementary Figures 1 and 2).

3.1 Behavior of a Δ*pgi* Δ*zwf* mutant

166   Cell growth was compared for strains M2 and M4, a Δ*pgi* Δ*zwf* mutant in rich medium

167   supplemented with various carbon sources (Figure 2). Maximum specific growth rates for each

168   combination of strain and carbon supplement were also calculated (Table II).   As expected,

169 growth of strain M2 was similar for all conditions tested, with similar lag phases and maximum

170 specific growth rates. Final cell densities were lower when strain M2 was fed D-glucose, likely

171 due to increased production of acetate (Figure 3), which has been shown to inhibit cell growth

172 (Roe et al., 1998). Growth of strain M4 was also similar for all conditions tested with the

173 exception of glycerol-supplemented cultures, which displayed a significant lag in growth of

174 approximately 24 hours. We hypothesize that this lag corresponds to depletion of the metabolic

175 precursors provided by LB and a metabolic shift towards gluconeogenic metabolism for growth

176 on glycerol. Maximum growth rate, lag time, and final cell densities are similar for strain M4 in

177 the presence of L-arabinose, glycerol, and D-xylose regardless of whether D-glucose was

178 supplemented in the growth medium, indicating that substrate consumption was similar in the

179 presence or absence of D-glucose. Overall, maximum growth rates of strain M4 were

180 approximately 70% that of strain M2.

181 Concentrations of D-glucose, alternative carbon source, and acetate were measured for each

182 strain/carbon supplement combination as a function of time (Figure 3). As expected, the

183 presence of D-glucose prevents consumption of the alternative carbon source in strain M2 via

184 catabolite repression. In contrast, the deletion of *pgi* and *zwf* prevents consumption of D-glucose

185 in strain M4. Interestingly, deletion of *pgi* and *zwf* appears to eliminate catabolite repression in

186 strain M4, as the presence of D-glucose does not preclude consumption of the alternative carbon

187 source in this strain.

188 This phenomenon is perhaps due to intracellular buildup of glucose-6-phosphate. Catabolite

189 repression is mediated by cyclic AMP (cAMP), which is synthesized by adenylate cylcase.

190 Adenylate cylcase is activated via phosphorylation by EIIA^{Glc}, but this phosphorylation can only

191 occur if EIIA^{Glc} has a phosphate group to donate. When glucose is being actively imported

192 through the PTS system, EIIA^{Glc} donates its phosphate to the incoming glucose, resulting in a

193 mostly unphosphorylated population of EIIA^{Glc}, inactive adenylate cyclase, and a low

194 concentration of cAMP. The absence or depletion of glucose from the culture medium leads to a

195 buildup of phosphorylated EIIA^{Glc}, activation of adenylate cyclase, and an increase in cAMP

196 concentration, eventually leading to the expression of catabolite-repressed genes such as *araBAD*

197 and *xylAB*, which are important for arabinose and xylose metabolism, respectively. Thus,

198 catabolite repression occurs when there is active glucose flux into the cell, not simply when

199 glucose is present in the medium. In a Δ*pgi* Δ*zwf* mutant, the accumulation of glucose-6-

200 phosphate quickly eliminates glucose flux into the cell, resulting in derepression of genes

201 normally repressed in the presence of glucose.

202 **3.2 D-glucaric acid production in a Δ*pgi* Δ*zwf* mutant**

203 D-glucaric acid, a dicarboxylic organic acid, is a naturally occurring product which has been

204 investigated for a variety of potential applications. A biosynthetic pathway to D-glucaric acid

205 from D-glucose has been constructed in *E. coli* (Moon et al., 2009). This pathway begins with

206 glucose-6-phosphate, which is converted to *myo*-inositol-1-phosphate by *myo*-inositol-1-

207 phosphate synthase (INO1). *Myo*-inositol-1-phosphate is then dephosphorylated by an

208 endogenous phosphatase to yield *myo*-inositol, which is oxidized to D-glucuronic acid by *myo*-

209 inositol oxygenase (MIOX). Finally, D-glucuronic acid is oxidized to D-glucaric acid by uronate

210 dehydrogenase (Udh). Because production of D-glucaric acid requires glucose-6-phosphate, we

211 hypothesized that the yield of D-glucaric acid could be increased significantly in a Δ*pgi* Δ*zwf*

212 strain.

213 In supplemented LB, D-glucaric acid yield on glucose is increased in the Δ*pgi* Δ*zwf* mutant

214 nearly 18-fold over an unmutated control supplemented with L-arabinose or D-xylose, while

215 yield is increased approximately 9-fold in the Δ*pgi* Δ*zwf* strain supplemented with glycerol

216 (Table III). Additionally, D-glucaric acid titers are significantly higher in the Δ*pgi* Δ*zwf* mutant

217 (Figure 4 and Table III). We hypothesize that deletion of *pgi* and *zwf* results in higher glucose-6-

218 phosphate pools, allowing INO1 to operate much closer to its maximum activity, which leads to

219 increased flux through the D-glucaric acid pathway.

220 To simulate a lean medium that might be obtained from the hydrolysis of lignocellulosic

221 biomass, the Δ*pgi* Δ*zwf* strain was also tested in a modified MOPS minimal medium containing

222 10 g/L D-glucose and 6 g/L L-arabinose. Although the strain grew more slowly under these

223 conditions, D-glucaric acid titers of 0.40 ± 0.02 g/L were obtained, nearly as much as observed

224 in supplemented LB (Figure 5). The yield of D-glucaric acid from glucose was 47%; however,

225 approximately 0.2 g/L of *myo*-inositol was also produced, bringing the total yield of G6P-derived

226 products to 71%. *Myo*-inositol is produced from G6P as an intermediate during D-glucaric acid

227 production and has previously been observed to build up in the culture medium under some

228 conditions (Moon et al., 2009). Modified MOPS medium containing D-xylose and D-glucose

229 was also tested, but no growth of the Δ*pgi* Δ*zwf* mutant was observed, possibly due to stronger

230 residual catabolite repression of *xylAB* in minimal medium. In modified MOPS medium

231 supplemented with D-xylose alone, M6 does not produce glucaric acid or *myo*-inositol,

232 consistent with the expectation that glucaric acid can only be derived from glucose in a Δ*pgi*

233 Δ*zwf* mutant (Supplementary Table 1).

4. Discussion

235 Traditionally, the main focus of metabolic engineering projects has been on increasing the

236 final titer of a product of interest, and this approach has been widely successful for high-value

237 compounds such as pharmaceutical intermediates and therapeutic proteins. However, increasing

238   titers alone may not be sufficient for low-margin, high-volume bioproducts such as commodity

239   chemicals. In these cases, product yield becomes an important process consideration, as raw

240   material costs can be a large percentage of the manufacturing costs. Strategies which are able to

241   increase product yield without sacrificing titer would be valuable tools for the metabolic

242   engineer.

243   The use of renewable feedstocks such as lignocelluosic biomass for biochemical production

244   presents an interesting opportunity for increasing the yield of biochemicals derived directly from

245   glucose: in addition to glucose, lignocellulosic biomass contains several other fermentable sugars

246   (e.g., xylose and arabinose) which may be used for biomass formation while reserving glucose

247   solely for product generation. Because wild-type *E. coli* preferentially consumes glucose, strain

248   engineering is necessary to shift the cell's preference towards alternative carbon sources. In this

249   work, we characterized the carbon source preference of a Δpgi Δzwf mutant and explored its

250   ability to improve the yield of D-glucaric acid on D-glucose.

251   As expected, deletion of *pgi* and *zwf* eliminates the cell's ability to consume D-glucose for

252   biomass formation. Catabolite repression is eliminated in this strain as well, as the Δpgi Δzwf

253   mutant is able to consume L-arabinose, glycerol, and D-xylose in the presence of D-glucose.

254   Because glucose-mediated catabolite repression occurs when glucose transport into the cell is

255   high, we believe that intracellular buildup of glucose-6-phosphate in the Δpgi Δzwf mutant leads

256   to significantly reduced glucose transport, alleviating catabolite repression.   Interestingly,

257   introduction of the D-glucaric acid pathway, which should draw down intracellular glucose-6-

258   phosphate pools, does not appear to affect the uptake of alternative carbon sources in the

259   presence of D-glucose. We speculate that glucose influx in the presence of INO1 is not high

260   enough to significantly reduce the levels of phosphorylated EIIA$^{\text{Glc}}$ to result in activation of

261   catabolite repression. Because the threshold rate of glucose import necessary for activation of

262   catabolite repression is unknown, efforts to further increase the activity of INO1 or to introduce

263   more active glucose consumption pathways should proceed with caution to avoid reactivation of

264   catabolite repression.

5. Conclusions

266   In this work, we investigate the behavior of a Δpgi Δzwf mutant and its ability to utilize

267   alternative carbon sources for cell growth while reserving D-glucose for product formation. This

268   strain was able to consume L-arabinose, glycerol, and D-xylose even in the presence of D-

269   glucose, and yields of D-glucaric acid on D-glucose were increased 9- to 18-fold in the Δpgi

270   Δzwf strain.   Additionally, product titers were also increased, as the initial D-glucaric acid

271   pathway enzyme was no longer in competition with glycolytic enzymes for glucose-6-phosphate.

272   Furthermore, the Δpgi Δzwf mutant exhibits similar yield increases in minimal medium,

273   suggesting the strain's potential in an industrial setting; however, additional investigation is

274   necessary to fully characterize the strain's robustness. These gains in product yield should easily

275   translate to other bioproducts derived from D-glucose, and it is hoped that this strain will help

276   improve the process economics of these value-added biochemicals.

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284 **References**

285 Amann E, Brosius J. 1985. "ATG vectors" for regulated high-level expression of cloned genes in

286   Escherichia coli. *Gene.* **40**:183–190.

287 Baba T, Ara T, Hasegawa M, Takai Y, Okumura Y, Baba M, Datsenko KA, Tomita M, Wanner

288   BL, Mori H. 2006. Construction of Escherichia coli K-12 in-frame, single-gene knockout

289   mutants: the Keio collection. *Mol. Syst. Biol.* **2**:2006.0008.

290 Balderas-Hernández VE, Hernández-Montalvo V, Bolívar F, Gosset G, Martínez A. 2011.

291   Adaptive evolution of Escherichia coli inactivated in the phosphotransferase system operon

292   improves co-utilization of xylose and glucose under anaerobic conditions. *Appl. Biochem.*

293   *Biotechnol.* **163**:485–496.

294 Chassagnole C, Noisommit-Rizzi N, Schmid JW, Mauch K, Reuss M. 2002. Dynamic modeling

295   of the central carbon metabolism of Escherichia coli. *Biotechnol. Bioeng.* **79**:53–73.

296   http://doi.wiley.com/10.1002/bit.10288.

297 Curran KA, Alper H. 2012. Expanding the chemical palate of cells by combining systems

298   biology and metabolic engineering. *Metab. Eng.* **14**:289–297.

299 Datsenko KA, Wanner BL. 2000. One-step inactivation of chromosomal genes in Escherichia

300   coli K-12 using PCR products. *Proc. Natl. Acad. Sci. U.S.A* **97**:6640–6645.

301 Gonçalves GAL, Prazeres DMF, Monteiro GA, Prather KLJ. 2013. De novo creation of

302   MG1655-derived E. coli strains specifically designed for plasmid DNA production. *Appl.*

303   *Microbiol. Biotechnol.* **97**:611–620.

304   Hernández-Montalvo V, Martínez A, Hernández-Chavez G, Bolivar F, Valle F, Gosset G. 2003.

305   Expression of galP and glk in a Escherichia coli PTS mutant restores glucose transport and

306   increases glycolytic flux to fermentation products. *Biotechnol. Bio* **83**:687–694.

307   Johnson DT, Taconi KA. 2009. The glycerin glut: options for the value-added conversion of

308   crude glycerol resulting from biodiesel production. *Environ. Prog.* **26**:338–348.

309   Joyce BL, Stewart CN. 2012. Designing the perfect plant feedstock for biofuel production: using

310   the whole buffalo to diversify fuels and products. *Biotechnol. Adv.* **30**:1011–1022.

311   Kogure T, Wakisaka N, Takaku H, Takagi M. 2007. Efficient production of 2-deoxy-scyllo-

312   inosose from D-glucose by metabolically engineered recombinant Escherichia coli. *J.*

313   *Biotechnol* **129**:502–509.

314   Lee SY. 1996. Bacterial Polyb ydroxyalkanoates **49**:1–14.

315   Moon TS, Yoon SH, Lanza AM, Roy-Mayhew JD, Prather KLJ. 2009. Production of glucaric

316   acid from a synthetic pathway in recombinant Escherichia coli. *Appl. Environ. Microbiol.*

317   **75**:589–595.

318   Pandey RP, Malla S, Simkhada D, Kim BG, Sohng JK. 2013. Production of 3-o-xylosyl

319   quercetin in Escherichia coli. *Appl. Microbiol. Biotechnol.* **97**:1889–1901.

320   Roe AJ, Mclaggan D, Davidson IAN, Byrne CO, Booth IR, Laggan DMC, Davidson IAN. 1998.

321   Perturbation of anion balance during inhibition of growth of Escherichia coli by weak acids.

322   *J. Bacteriol.* **180**:767–772.

323   Rogers P, Chen J, Zidwick MJO. 2006. The Prokaryotes. New York, NY: Springer New York.

324   http://www.springerlink.com/index/10.1007/0-387-30741-9.

325   Sambrook J, Russell DW. 2001. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor

326   Laboratory Press, Cold Spring Harbor.

327   Shiue E, Prather KLJ. 2014. Improving D-glucaric acid production from myo-inositol in E. coli

328   by increasing MIOX stability and myo-inositol transport. *Metab. Eng.* **22**:22–31.

329   Solomon K V, Moon TS, Ma B, Sanders TM, Prather KLJ. 2013. Tuning primary metabolism

330   for heterologous pathway productivity. *ACS Synth. Biol.* **2**:126–135.

331   Wang D, Li Q, Yang M, Zhang Y, Su Z, Xing J. 2011. Efficient production of succinic acid from

332   corn stalk hydrolysates by a recombinant Escherichia coli with ptsG mutation. *Process*

333   *Biochem.* **46**:365–371.

334   Werpy T, Petersen G. 2004. Top value added chemicals from biomass, volume 1: results of

335   screening for potential candidates from sugars and synthesis gas. *U. S. Dep. Energy*. U.S.

336   Department of Energy, Washington, DC.

337   Yoon SH, Moon TS, Iranpour P, Lanza AM, Prather KLJ. 2009. Cloning and characterization of

338   uronate dehydrogenases from two pseudomonads and Agrobacterium tumefaciens strain

339   C58. *J. Bacteriol.* **191**:1565–1573.

Table I: *E. coli* strains, plasmids, and oligonucleotides used

Name	Relevant Genotype	Reference
Strains		
DH10B	F⁻ *mcrA Δ(mrr-hsdRMS-mcrBC) φ80lacZΔM15 ΔlacX74 recA1 endA1 araD139 Δ(ara, leu)7697 galU galK λ-rpsL nupG*	Life Technologies (Carlsbad, CA)
JW1841-1	F⁻, *Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ⁻, Δzwf-777::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	CGSC #9537 (Baba et al., 2006)
MG1655	F⁻ λ⁻ *ilvG frb- 50 rph-1*	ATCC #700926
M2	MG1655(DE3) *ΔendA ΔrecA*	(Shiue and Prather, 2014)
M2-2	MG1655(DE3) *ΔendA ΔrecA ΔgudD ΔuxaC*	(Shiue and Prather, 2014)
M3	MG1655 *ΔendA ΔrecA Δpgi*	(Gonçalves et al., 2013)
M4	MG1655(DE3) *ΔendA ΔrecA Δpgi Δzwf*	This study
M5	MG1655(DE3) *ΔendA ΔrecA Δpgi Δzwf ΔuxaC*	This study
M6	MG1655(DE3) *ΔendA ΔrecA Δpgi Δzwf ΔuxaC ΔgudD*	This study
Plasmids		
pCP20	Repᵃ, Ampᴿ, Cmᴿ, FLP recombinase expressed by λ pᵣ under control of λ cI857	CGSC #7629
pKD13	R6Kγ *ori*, Ampᴿ, *kan*	CGSC #7633
pKD46	R101 *ori*, *repA101*ᵃ, Ampᴿ, *araC, araBp-λᵧ-λᵦ-λₑₓₒ*	CGSC #7739
pKD46recA	R101 *ori*, *repA101*ᵃ, Ampᴿ, *araC, araBp-λᵧ-λᵦ-λₑₓₒ, recA*	(Solomon et al., 2013)
pRSFDuet-1	pRSR1030 *ori,* lacI, Kanᴿ	EMD4 Biosciences (Darmstadt, Germany)
pTrc99A	pBR322 *ori,* Ampᴿ	(Amann and Brosius, 1985)
pRSFD-IN	pRSFDuet-1 with INO1 inserted into the EcoRI and HindIII sites	(Moon et al., 2009)
pTrc-Udh	pTrc99A with Udh from *Pseudomonas syringae* inserted into the NcoI and HindIII sites	(Moon et al., 2009; Yoon et al., 2009)
pRSFD-IN-Udh	pRSFD-IN with Udh inserted into the MfeI and XhoI sites	This study
pTrc-SUMO-MIOX	pTrc99A with SUMO-MIOX	(Shiue and Prather, 2014)

Oligonucleotides

pKD13_gudD_fwd
pKD13_gudD_rev
pKD13_uxaC_fwd
pKD13_uxaC_rev

[a] All oligonucleotides purchased from Sigma-Genosys (St. Louis, MO). Homologous sequences used for recombination are underlined.

Table II: Maximum growth rates of various strains in rich medium supplemented with various carbon sources.

Strain[*]	Alternative Carbon Source	Glucose	μ_{max} (hr^{-1})
M2	L-Arabinose	-	1.00 ± 0.01
		+	1.01 ± 0.06
	Glycerol	-	0.98 ± 0.02
		+	0.97 ± 0.04
	D-Xylose	-	1.00 ± 0.03
		+	0.87 ± 0.20
M4	L-Arabinose	-	0.65 ± 0.01
		+	0.66 ± 0.02
	Glycerol	-	0.66 ± 0.05
		+	0.68 ± 0.01
	D-Xylose	-	0.70 ± 0.01
		+	0.67 ± 0.01

[*] **M2:** MG1655(DE3) *ΔendA ΔrecA;* **M4:** MG1655(DE3) *ΔendA ΔrecA Δpgi Δzwf*

Table III: D-glucaric acid yields on D-glucose for various carbon sources.

Strain[*]	Carbon	D-Glucaric Acid Titer (g/L)	Yield on D-Glucose (g/g)
M2-2	L-Arabinose in LB	0.13 ± 0.01	0.044 ± 0.002
	Glycerol in LB	0.20 ± 0.02	0.052 ± 0.009
	D-Xylose in LB	0.13 ± 0.01	0.039 ± 0.002
M6	L-Arabinose in LB	0.50 ± 0.01	0.76 ± 0.13
	Glycerol in LB	0.81 ± 0.10	0.44 ± 0.04
	D-Xylose in LB	1.19 ± 0.08	0.73 ± 0.03
	L-Arabinose in MOPS	0.40 ± 0.02	0.47 ± 0.25

[*] **M2-2:** MG1655(DE3) *ΔendA ΔrecA ΔgudD ΔuxaC;* **M6:** MG1655(DE3) *ΔendA ΔrecA Δpgi Δzwf ΔuxaC ΔgudD*

Figures

Figure 1: Glucose utilization pathways in *E. coli*.

Figure 2: Growth curves for strains M2 (top row) and M4 (bottom row) in rich medium supplemented with L-arabinose ("Ara", triangles), glycerol ("Gly", circles), and D-xylose ("Xyl", squares) with D-glucose ("Glc") absent (open points) or present (filled points). M2: MG1655(DE3) *ΔendA recA*; M4: MG1655(DE3) *ΔendA ΔrecA Δpgi Δzwf*.

Figure 3: Carbon source and acetate ("Act", circles) concentrations in rich medium cultures of M2 (filled points, solid line) and M4 (open points, dotted lines) supplemented with L-arabinose ("Ara", triangles), glycerol ("Gly", inverted triangles), and D-xylose ("Xyl", diamonds) with D-glucose ("Glc", squares) absent (left column) or present (right column). M2: MG1655(DE3) *ΔendA ΔrecA*; M4: MG1655(DE3) *ΔendA ΔrecA Δpgi Δzwf*.

Figure 4: D-glucaric acid production ("Gla", circles) from D-glucose in strains M2-2 and M6 in rich medium. Cultures contained strain M2-2 or M6 harboring pRSFD-IN-Udh and pTrc-SUMO-MIOX and were grown in LB supplemented with D-glucose ("Glc", squares) and L-arabinose ("Ara", triangles), glycerol ("Gly", inverted triangles), or D-xylose ("Xyl", diamonds). M2-2: MG1655(DE3) *ΔendA ΔrecA ΔgudD ΔuxaC* (filled points, solid line); M6: MG1655(DE3) *ΔendA ΔrecA ΔgudD ΔuxaC Δpgi Δzwf* (open points, dotted line).

Figure 5: Growth and D-glucaric acid ("Gla", circles) production from D-glucose in strain M6 in lean medium. Cultures contained M6 harboring pRSFD-IN-Udh and pTrc-SUMO-MIOX and were grown in modified MOPS minimal medium supplemented with 6 g/L L-arabinose ("Ara", triangles) and 10 g/L D-glucose ("Glc", squares). M6: MG1655(DE3) *ΔendA ΔrecA ΔgudD ΔuxaC Δpgi Δzwf*.

Graphical TOC: Knockout of pgi and zwf from E. coli prevents the cell from using glucose for biomass production, allowing the carbon to be diverted towards bioproducts of interest (e.g., D-glucaric acid). Alternative carbon sources (e.g., D-xylose, glycerol, and L-arabinose), which may be derived from biomass hydrolysis, can be fed for biomass formation.

Figure 1

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Figure 2

Figure 3

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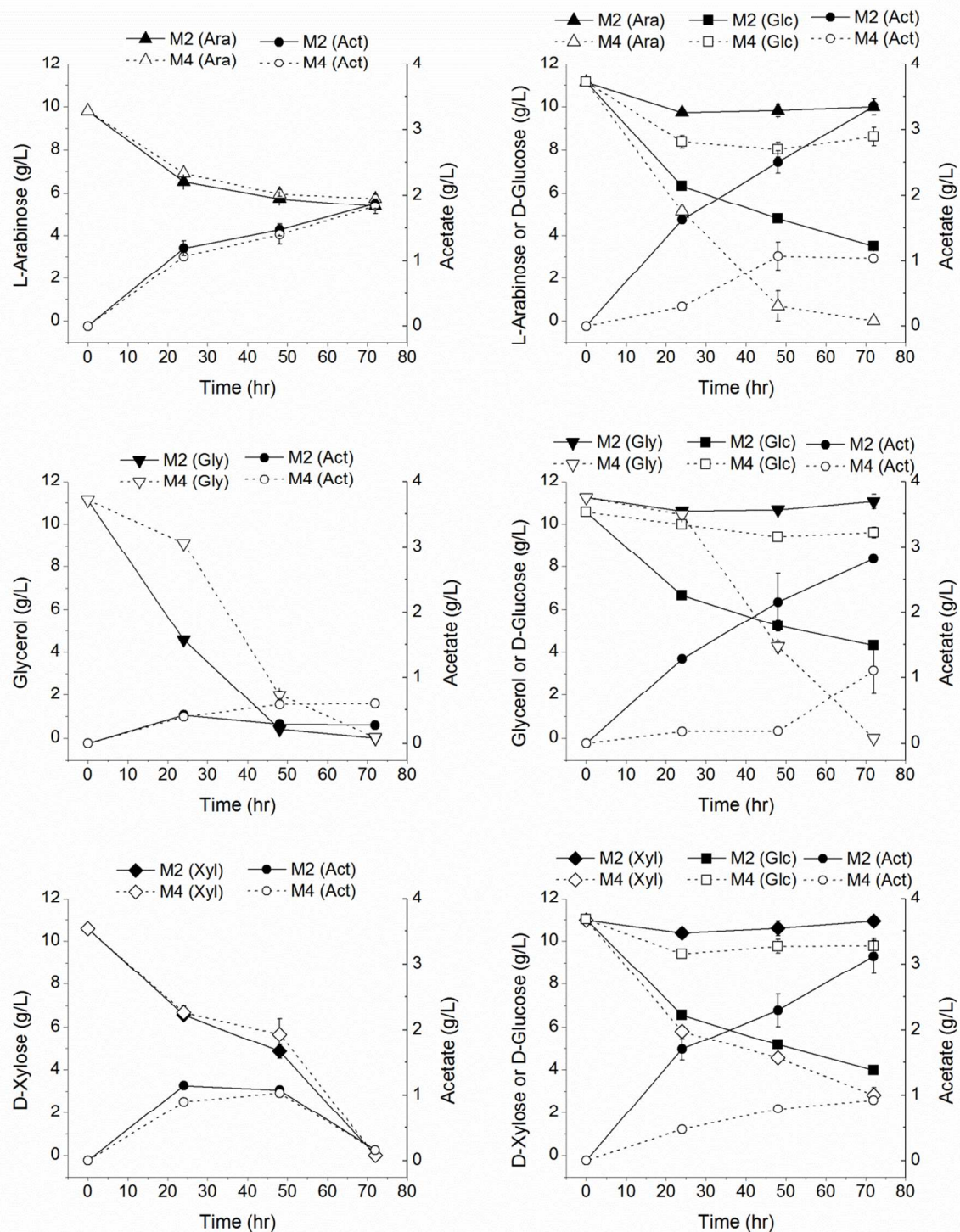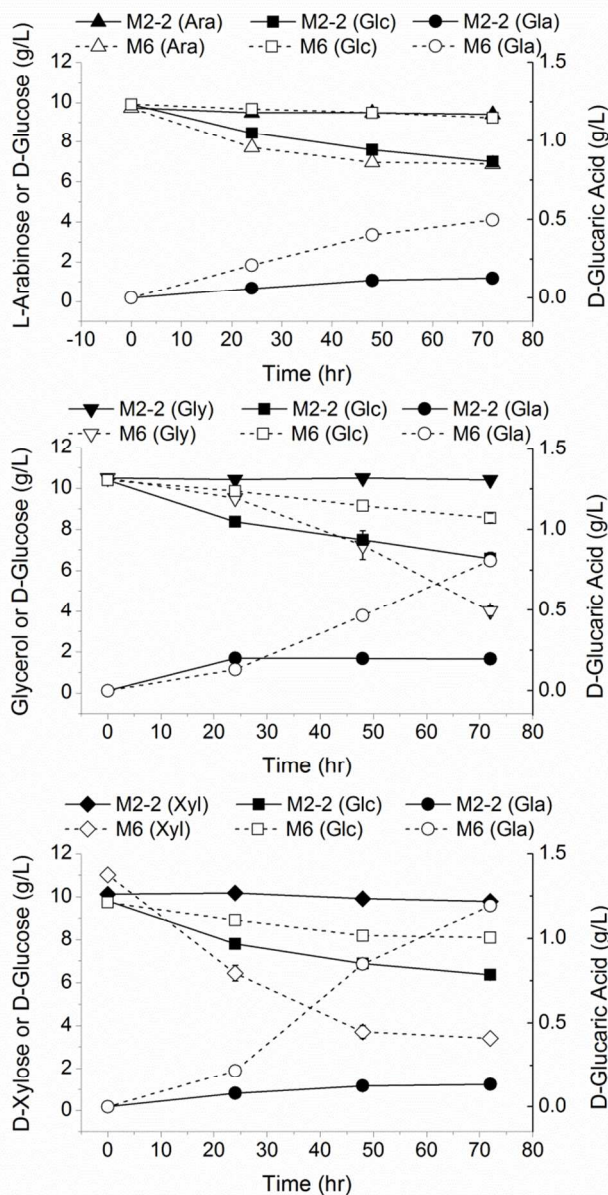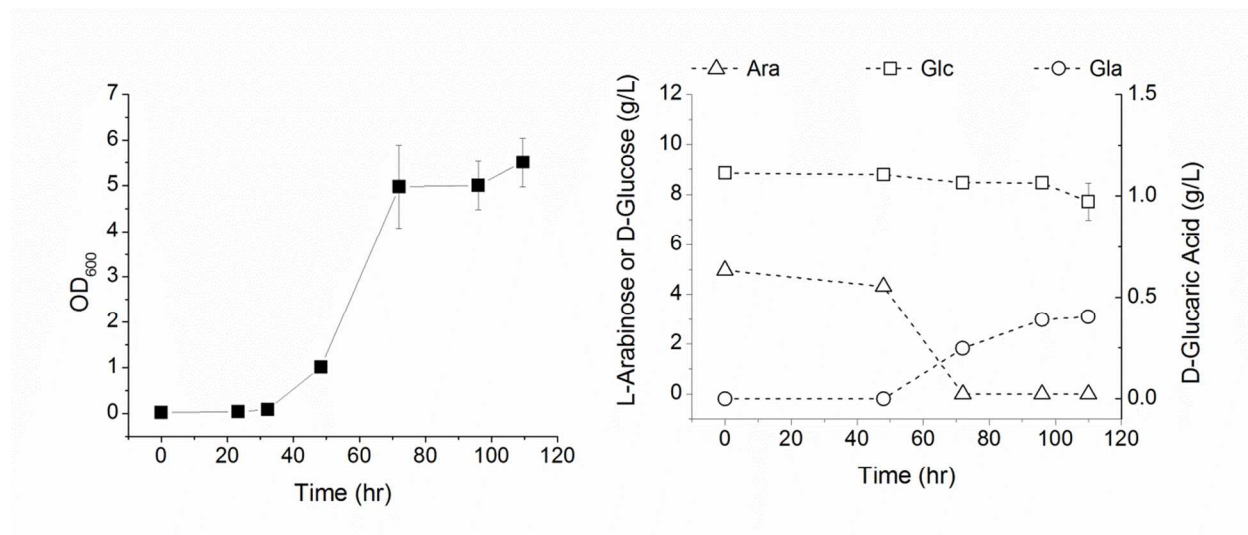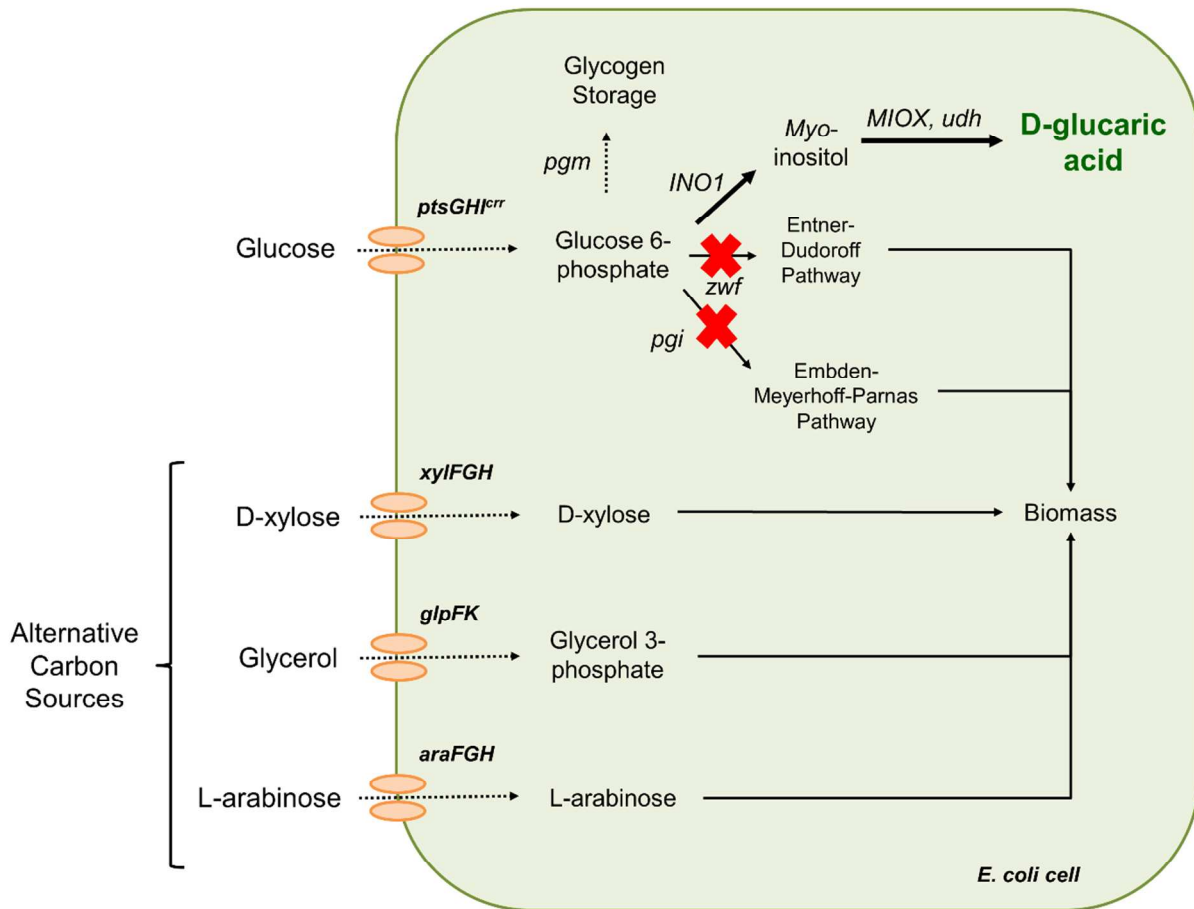3
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Figure 4

Figure 5

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Graphical TOC

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Oligonucleotides	5' → 3' Sequence[a]
pKD13_gudD_fwd	TCCCCGGCTGGACCTTTGACCGTAAACGTCCGTTTTCGGCCGTCATTGATTCTGAAAAAGGACATAAATCTGTCAAACATGAGAATTAATTCC
pKD13_gudD_rev	CAACAGGCTATTTTGCGTTTAGCATCAGTCTCAAACCGGCTCCAGATAGAGCCGGTTTTGGTTTTCTGTCGTGTAGGCTGGAGCTGCTTC
pKD13_uxaC_fwd	AATTGGTGTGATAACTTTGTCAGCATCGCACCATAAGCAAGCTAGCTCACTCGTTGAGAGGAAGACGAAACTGTCAAACATGAGAATTAATTCC
pKD13_uxaC_rev	AAATCTGCTAAAGCGACCGCGACGTTATCCAGCGCATGGATCTTGATGTATTGCATATCAACCCCAGACCGTGTAGGCTGGAGCTGCTTC

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		+	0.66 ± 0.02
	Glycerol	-	0.66 ± 0.05
		+	0.68 ± 0.01
	D-Xylose	-	0.70 ± 0.01
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M6	L-Arabinose in LB	0.50 ± 0.01	0.76 ± 0.13
	Glycerol in LB	0.81 ± 0.10	0.44 ± 0.04
	D-Xylose in LB	1.19 ± 0.08	0.73 ± 0.03
	L-Arabinose in MOPS	0.40 ± 0.02	0.47 ± 0.25

[*] **M2-2:** MG1655(DE3) *ΔendA ΔrecA ΔgudD ΔuxaC;* **M6:** MG1655(DE3) *ΔendA ΔrecA Δpgi Δzwf ΔuxaC ΔgudD*

Glucose utilization pathways in E. coli
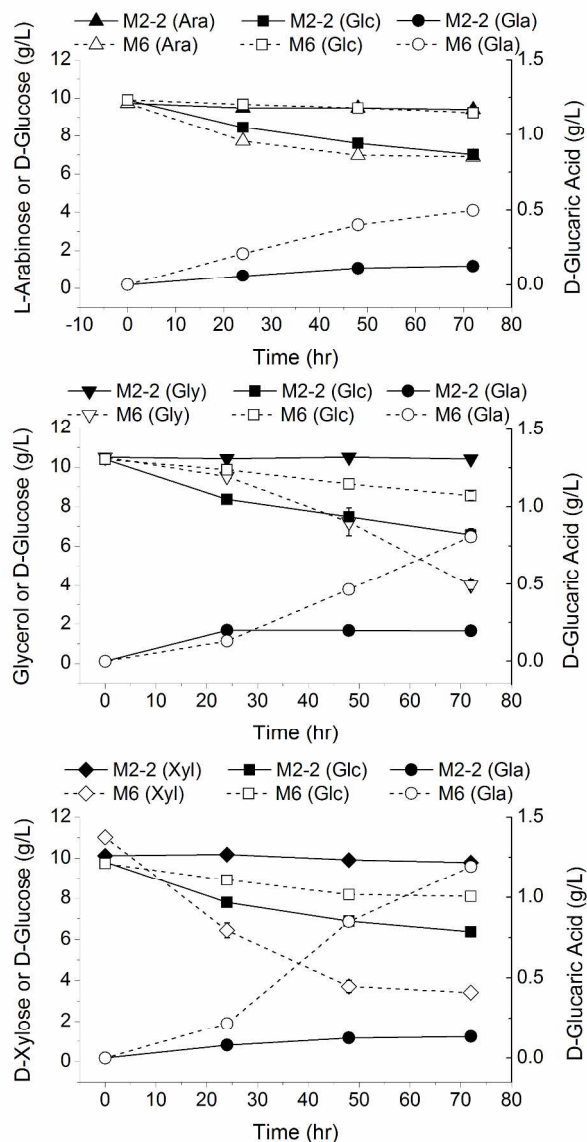168x143mm (300 x 300 DPI)

Growth curves for strains M2 (top row) and M4 (bottom row) in rich medium supplemented with L-arabinose ("Ara", triangles), glycerol ("Gly", circles), and D-xylose ("Xyl", squares) with D-glucose ("Glc") absent (open points) or present (filled points). M2: MG1655(DE3) ΔendA recA; M4: MG1655(DE3) ΔendA ΔrecA Δpgi Δzwf.
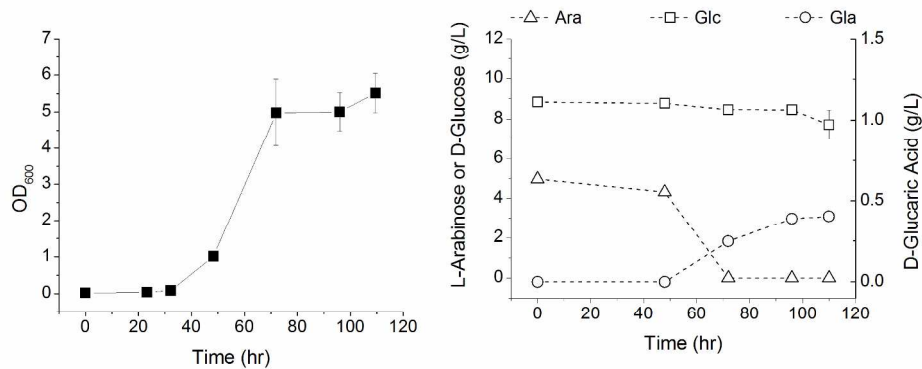271x203mm (300 x 300 DPI)
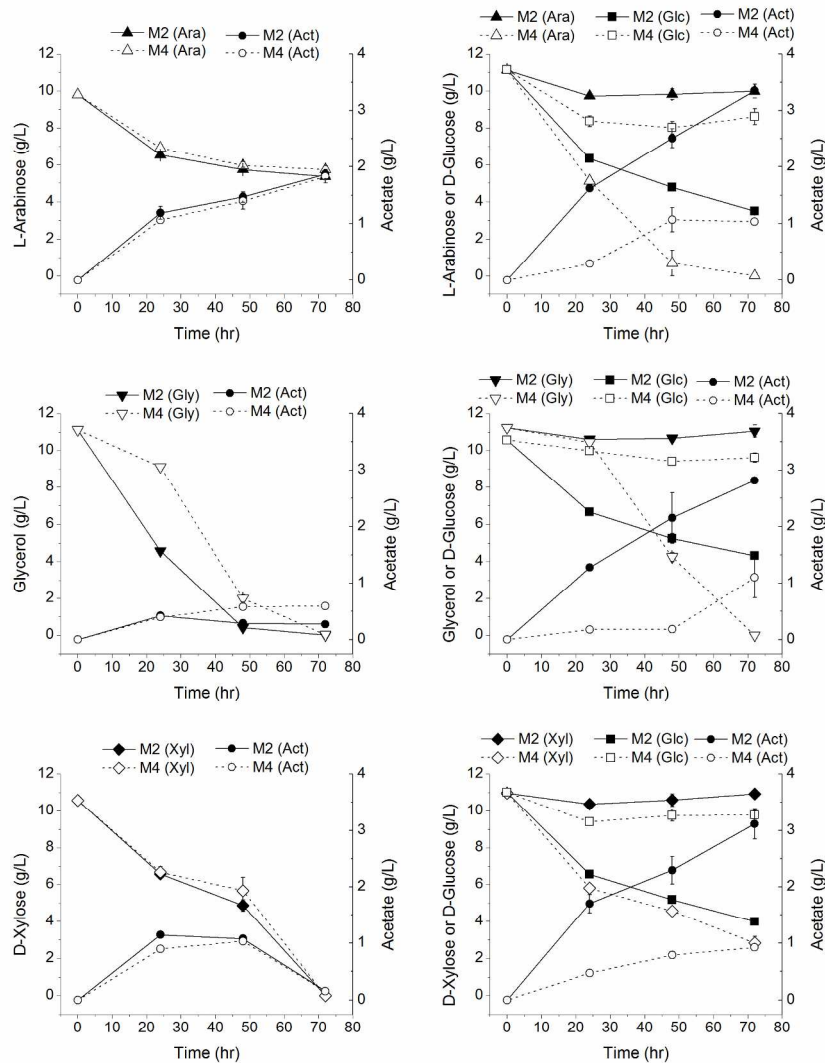
Carbon source and acetate ("Act", circles) concentrations in rich medium cultures of M2 (filled points, solid line) and M4 (open points, dotted lines) supplemented with L-arabinose ("Ara", triangles), glycerol ("Gly", inverted triangles), and D-xylose ("Xyl", diamonds) with D-glucose ("Glc", squares) absent (left column) or present (right column). M2: MG1655(DE3) ΔendA ΔrecA; M4: MG1655(DE3) ΔendA ΔrecA Δpgi Δzwf.
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D-glucaric acid production ("Gla", circles) from D-glucose in strains M2-2 and M6 in rich medium. Cultures contained strain M2-2 or M6 harboring pRSFD-IN-Udh and pTrc-SUMO-MIOX and were grown in LB supplemented with D-glucose ("Glc", squares) and L-arabinose ("Ara", triangles), glycerol ("Gly", inverted triangles), or D-xylose ("Xyl", diamonds). M2-2: MG1655(DE3) ΔendA ΔrecA ΔgudD ΔuxaC (filled points, solid line); M6: MG1655(DE3) ΔendA ΔrecA ΔgudD ΔuxaC Δpgi Δzwf (open points, dotted line).
178x341mm (300 x 300 DPI)

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Growth and D-glucaric acid ("Gla", circles) production from D-glucose in strain M6 in lean medium. Cultures contained M6 harboring pRSFD-IN-Udh and pTrc-SUMO-MIOX and were grown in modified MOPS minimal medium supplemented with 6 g/L L-arabinose ("Ara", triangles) and 10 g/L D-glucose ("Glc", squares). M6: MG1655(DE3) ΔendA ΔrecA ΔgudD ΔuxaC Δpgi Δzwf.
250x108mm (300 x 300 DPI)
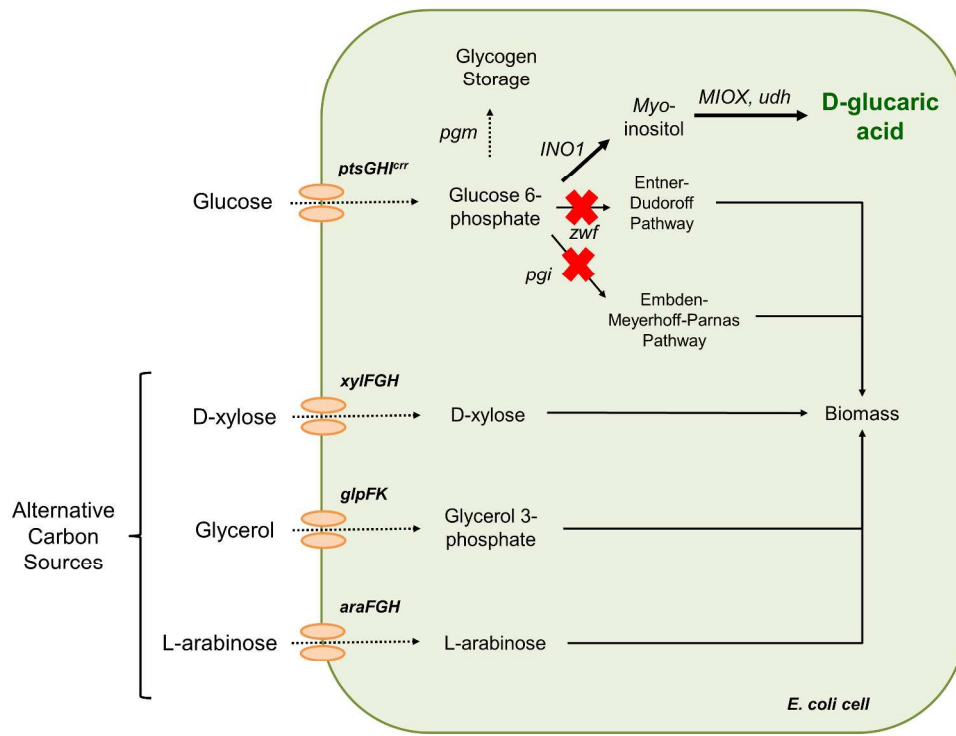
Knockout of pgi and zwf from E. coli prevents the cell from using glucose for biomass production, allowing the carbon to be diverted towards bioproducts of interest (e.g., D-glucaric acid). Alternative carbon sources (e.g., D-xylose, glycerol, and L-arabinose), which may be derived from biomass hydrolysis, can be fed for biomass formation.
293x227mm (300 x 300 DPI)

Supporting Information for *Improving Product Yields on D-Glucose in Escherichia coli via Knockout of pgi and zwf and Feeding of Supplemental Carbon Sources*

Eric Shiue, Irene M. Brockman, and Kristala L. J. Prather

Supplementary Methods

Supplementary Table 1: Glucaric acid production by M2-2 and M6 in modified MOPS minimal medium with 10 g/L xylose

Supplementary Figure 1: Growth of strains M2 and M4 in M9 minimal medium supplemented with D-glucose, D-xylose, or L-arabinose

Supplementary Figure 2: Growth of strains M2 and M4 on M9 agar supplemented with D-glucose, D-xylose, or L-arabinose

Supplementary Methods

For glucaric acid production from xylose, cultures were grown in 250 mL baffled shake flasks containing a modified MOPS-buffered medium with 10 g/L D-xylose, 6 g/L NH_4Cl, 2 g/L K_2HPO_4, 2 mM $MgSO_4$, 0.1 mM $CaCl_2$, 40 mM MOPS, 4 mM tricine, 50 mM NaCl, 100 mM Bis-Tris, 134 μM EDTA, 31 μM $FeCl_3$, 6.2 μM $ZnCl_3$, 0.76 μM $CuCl_2$, 0.42 μM $CoCl_2$, 1.62 μM H_3BO_3, 0.081 μM $MnCl_2$, carbenicillin (100 μg/mL), and kanamycin (30 μg/ml). Seed cultures were started using a 1:100 dilution from LB and were grown at 30°C for 24 hours in modified MOPS. Working cultures were inoculated to an OD_{600} of 0.02 and induced at inoculation with 0.05 mM IPTG. Cultures were incubated at 30°C, 250 rpm, and 80% relative humidity for 120 hours. Samples were taken periodically, centrifuged to remove cell debris, and the supernatants analyzed for metabolite concentrations by HPLC.
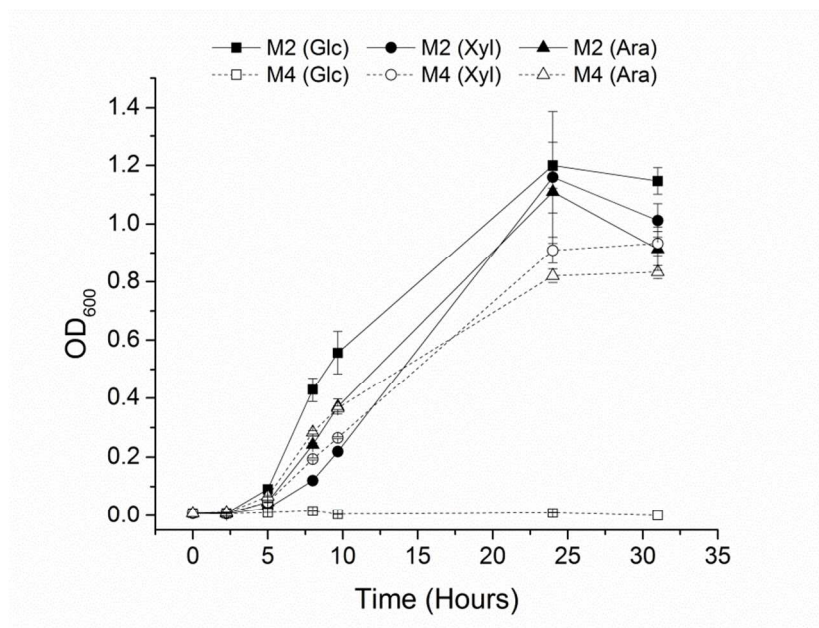
For growth measurements of M2 and M4, cultures were grown in M9 minimal medium supplemented with 4 g/L of the indicated carbon source (D-glucose, D-xylose, or L-arabinose). Seed cultures were started using a 1:100 dilution from LB into M9 with the appropriate carbon source. After growth at 37°C for 24 hours, these seed cultures were used to inoculate a working culture of M9 with the same carbon source for growth rate measurement. As the seed cultures for strain M4 did not grow in M9 + glucose, an M9 + xylose starter culture was used to seed the M9 + glucose culture for growth rate measurements. For growth on solid medium, M9 cultures at $OD_{600} \sim 0.2$ were diluted 10^3 or 10^4 and 100 μl was plated on M9 agar with the same carbon source. For strain M4, a culture in M9 + xylose was used for dilution and plating on M9 glucose agar, as the strain did not grow in glucose minimal medium seed cultures.

Supplementary Table 1: Glucaric acid production by M2-2 and M6 harboring plasmids pTrc-

SUMO-MIOX and pRSFD-IN-udh in modified MOPS minimal medium with 10 g/L xylose.

Glucaric acid and *myo*-inositol production were not detected in M6, as expected due to the

deletion of *pgi* and *zwf*, which prevents xylose flux toward glucose-6-phosphate. Peaks with

retention time corresponding to the glucaric acid standard were observed in M2-2 cultures but

were below the threshold for quantification. M2-2: MG1655(DE3) *ΔendA ΔrecA ΔgudD ΔuxaC*;

M6: MG1655(DE3) *ΔendA ΔrecA ΔgudD ΔuxaC Δpgi Δzwf*.

	M2-2			**M6**		
	OD	**glucaric acid (g/L)**	***myo*-inositol (g/L)**	**OD**	**glucaric acid (g/L)**	***myo*-inositol (g/L)**
72 hours	2.2 ± 0.7	n.d.	n.d.	0.25 ± 0.01	n.d.	n.d.
120 hours	6.6 ± 0.2	< 0.1	0.025 ± 0.02	6.6 ± 0.2	n.d.	n.d.

n.d. = not detected

Supplementary Figure 1: Growth of M2 (filled symbols) and M4 (open symbols) in M9

minimal supplemented with D-glucose ("Glc", squares), D-xylose ("Xyl", circles), or L-

arabinose ("Ara", triangles). M2: MG1655(DE3) *ΔendA ΔrecA*; M4: MG1655(DE3) *ΔendA*

ΔrecA Δpgi Δzwf.

Supplementary Figure 2: Growth of M2 and M4 on M9 agar supplemented with D-glucose, D-xylose, or L-arabinose. (A) M9 agar plates after growth for 48 hours at 37° C. No growth was observed for strain M4 on glucose. Carbon sources (left-right): D-glucose, D-xylose, L-arabinose. Strain and dilution (top to bottom): M2 at 10^4 dilution, M4 at 10^4 dilution, M4 at 10^3 dilution. (B) Closer view of M4 plates at 10^3 dilution. Carbon source (left-right): D-glucose, D-xylose, L-arabinose. M2: MG1655(DE3) $\Delta endA \Delta recA$; M4: MG1655(DE3) $\Delta endA \Delta recA \Delta pgi \Delta zwf$.