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Escherichia coli HGT: Engineered for high glucose throughput even under slowly growing or resting conditions

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ABSTRACT

Aerobic production-scale processes are constrained by the technical limitations of maximum oxygen transfer and heat removal. Consequently, microbial activity is often controlled via limited nutrient feeding to maintain it within technical operability. Here, we present an alternative approach based on a newly engineered *Escherichia coli* strain. This *E. coli* HGT (high glucose throughput) strain was engineered by modulating the stringent response regulation program and decreasing the activity of pyruvate dehydrogenase. The strain offers about three-fold higher rates of cell-specific glucose uptake under nitrogen-limitation (0.6 g_{Glc} g_{CDW}^{-1} h^{-1}) compared to that of wild type, with a maximum glucose uptake rate of about 1.8 g_{Glc} g_{CDW}^{-1} h^{-1} already at a 0.3 h^{-1} specific growth rate. The surplus of imported glucose is almost completely available via pyruvate and is used to fuel pyruvate and lactate formation. Thus, *E. coli* HGT represents a novel chassis as a host for pyruvate-derived products.

1. Introduction

Aerobic industrial production processes using microbes are predominantly run in fed-batch mode to achieve the highest product titers, substrate conversion yields and product purity. After the exponential phase of biomass formation, such processes typically need to induce low microbial activities to meet the technical limitations in maximum oxygen transfer (often in the range of 150–180 mmol L^{-1} h^{-1}) and heat removal (Takors, 2012). As an easy-to-implement measure, operators in industrial plants often introduce carbon (glucose)-limiting feeds, which in turn also reduce product formation rates.

If process engineers were asked to list the characteristics of ideal producers, they would prefer strains that show high product conversion rates, even if they are limited in growth and oxygen uptake. In other words, cell-specific productivity and conversion yield should be high in non-growing (resting) or slowly growing cells.

Studying *Escherichia coli*, Chubukov and Sauer (2014) have shown that the limitation of nitrogen, phosphate, sulfur or magnesium yields different glucose conversion rates for non-growing cells, for example achieving biomass-specific glucose uptake rates of 0.083 and 0.162 g_{Glc} g_{CDW}^{-1} h^{-1} for the limitation of nitrogen and phosphate, respectively. In comparison, a documented value of maintenance for *E. coli* is only 0.057 g_{Glc} g_{CDW}^{-1} h^{-1} (Nielsen et al., 2003) derived from glucose-limited conditions and some authors have even detected lower values about 0.024 g_{Glc} g_{CDW}^{-1} h^{-1} (Taymaz-Nikerel et al., 2010). Indeed, proper phosphate-limitation may be a useful tool for industrial applications to install nutrient limitations without limiting elements of the target molecules, but it has inherent drawbacks that could severely hamper large-scale productions (Schuhmacher et al., 2014).

The question therefore arises as to whether *E. coli* strains can be specifically engineered to create a chassis for high glucose uptake even under non- or slowly-growing conditions.

Studies on pyruvate production by Zhu et al. (2008) have shown that reducing the activity of AceE (E1 component of pyruvate dehydrogenase complex, PDHC) is essentially beneficial for achieving high glucose uptake rates. Accordingly, likewise strains may serve as a starting point of strain engineering for providing enhanced amounts of pyruvate for downstream products. However, strain performance in large-scale is often hampered by regulation programs that are induced under such heterogeneous conditions. Recent studies by Löffler et al. (2016) revealed that stringent response plays a key role in large-scale fermentations, because related regulation programs are frequently switched on and off when circulating cells face heterogeneities in a production vessel. The stringent response is very complex (Fig. 1); activating and repressing different segments of microbial metabolism at the same time and closely networking with cAMP- and Cra-mediated control regimes (Hardiman et al., 2007; Johansson et al., 2000; Lemuth et al., 2008). GTP pyrophosphokinase is encoded by the *relA* gene and plays a dominant role, as it is responsible for production of the stringent response alarmone (p)ppGpp (from here on referred to as ppGpp).

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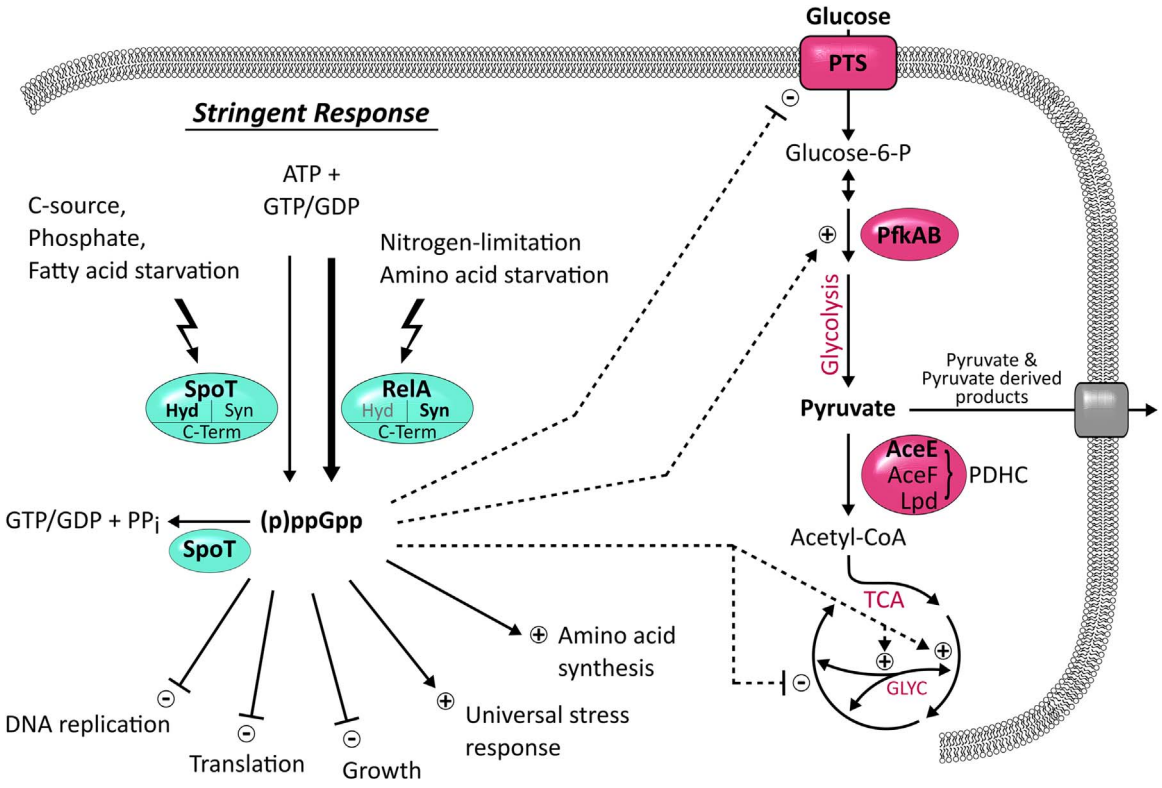

Fig. 1. Stringent response in *Escherichia coli*.

Nutritional depletion conditions in the environment are sensed by the two major factors of stringent response: RelA and SpoT. Intracellular signaling occurs via the alarmone (p)ppGpp, which is catalyzed from ATP and GTP or GDP, respectively. Activity of RelA is initiated at nitrogen-based stress conditions (Brown et al., 2014) and amino acid starvation (Haseltine and Block, 1973). SpoT is activated e.g. upon phosphate (Bougdour and Gottesman, 2007), carbon (Gentry and Cashel, 1996) or fatty acid starvation (Battesti and Bouveret, 2006). The two proteins share high structural similarity within their conserved N- and C-terminal (C-Term) domains, although the monofunctional RelA protein exerts only (p)ppGpp synthase activity, while SpoT has both abilities of (p)ppGpp synthesis (Syn) and hydrolysis (Hyd). Elevated (p)ppGpp levels have activating (plus symbol) or inhibitory (minus symbol) regulatory effects on various cellular mechanisms. Transcription profiling studies (Durfee et al., 2008; Traxler et al., 2008) suggest possible effects of (p)ppGpp on different sites of the central carbon metabolism (indicated with dashed arrows), like the PTS (phosphotransferase system), glycolysis (PfkAB: phosphofructokinase), TCA (tricarboxylic acid) cycle and GLYC (glyoxylate cycle).

Consequently, changing fundamental glucose uptake kinetics is a challenging goal that requires the careful modulation of regulatory signals, such that glucose uptake of *E. coli* is increased to provide a surplus for product formation that native regulatory programs would prevent. The present study demonstrates the engineering and kinetic characterization of *E. coli* HGT (high glucose throughput) as a novel chassis for exploiting additional glucose uptake to fuel product formation even under non- or slow-growth conditions.

2. Material and methods

2.1. Strains and plasmids

The laboratory wild type strain *Escherichia coli* MG1655 (Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures; #DSM-18039) was used in all experiments. This host strain and its derivatives as well as all plasmids and oligonucleotides used in this study are listed in Table 1. For cloning purposes, strains were grown either on tryptone-yeast extract (2×TY (Sambrook and Russell, 2001)) agar plates or in 5 mL 2×TY in glass reaction tubes, supplemented with the appropriate antibiotic at 50 μg mL^{-1}.

2.2. Scarless genome modifications

Chromosomal modifications of genes, without leaving any marker sequences in the genome, were accomplished by combining the pEMG and pACBSR genetic systems. The donor plasmid constructs (pEMG) were designed using classical cloning methods, as described in the deletion/replacement system protocol (Martínez-García and de Lorenzo, 2012) for pEMG+Δ*relA* and pEMG+*spoT*$^{[R290E;K292D]}$. Another pEMG plasmid construct, pEMG+*aceE*$^{[G267C]}$ was cloned using the Gibson one-step ISO assembly method (Gibson, 2011). Plasmid DNA was transfected into cells via electroporation. First, the pEMG donor plasmid was delivered into host cells. Due to a missing *pir* gene in the *E. coli* MG1655 hosts, *ori*R6K is unable to replicate, and thus the pEMG plasmid sequences were integrated into the host chromosome (Filutowicz et al., 1986). These co-integrates were resolved by introducing the pACBSR plasmid and inducing transcription of I-SceI and the λ Red genes with L-arabinose. The homing endonuclease, I-SceI, cuts the donor plasmid and generates double-strand breaks in flanking regions of the desired mutations. Products of the λ Red genes subsequently seal these linear fragments by homologous recombination with the chromosome (Herring et al., 2003). Plasmid sequences and genetic modifications were verified by Sanger sequencing (GATC, Konstanz, Germany). Amino acid positions in proteins of *E. coli* MG1655 derive from predicted protein sequences from the Universal Protein Resource Knowledgebase (UniProt Consortium, 2015). Both amino acid positions in SpoT, which were targets of genetic manipulation, were identified based on the UniProtKB entry with accession number P0AG25. The G267C amino acid substitution in AceE occurred spontaneously in *E. coli* tests causing a growth