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Adaptation for fast growth on glucose by differential expression of central carbon metabolism and *gal* regulon genes in an *Escherichia coli* strain lacking the phosphoenolpyruvate: carbohydrate phosphotransferase system

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Abstract

Phosphoenolpyruvate (PEP) is a key intermediate of cellular metabolism and a precursor of commercially relevant products. In *Escherichia coli* 50% of the glucose-derived PEP is consumed by the PEP:carbohydrate phosphotransferase system (PTS) for glucose transport. PTS, encoded by the *ptsHIcrr* operon, was deleted from JM101 to generate strain PB11 (PTS^-Glc^-). PB12, a mutant derived from PB11, grows faster than the parental strain on glucose (PTS^-Glc^+ phenotype). This strain can redirect some of the PEP not utilized by PTS into the high yield synthesis of aromatic compounds from glucose. Here, we report a comparative transcription analysis among these strains of more than 100 genes involved in central carbon metabolism during growth on glucose. It was found that in the PTS^- strains that have reduced glucose transport capacities, several genes encoding proteins with functions related to carbon transport and metabolism were upregulated. Therefore, it could be inferred that these strains synthesize autoinducers of these genes when sensing very low internal glucose concentrations, probably for scavenging purposes. This condition that is permanently present in the PTS^- strains even when growing in high glucose concentrations allowed the simultaneous utilization of glucose and acetate as carbon sources. It was found that the *gal* operon is upregulated in these strains, as well as the *aceBAK*, *poxB* and *acs* genes among others. In PB12, *glk*, *pgi*, the TCA cycle and certain respiratory genes are also upregulated. A mutation in *arcB* in PB12 is apparently responsible for the upregulation of the TCA cycle and certain respiratory genes.

Keywords: *Escherichia coli*; PTS^-; *arcA*; *arcB*; *galP*; *glk*; *pgi*; *rpoS*; RT–PCR; Starvation-stress response; TCA; Glyoxylate shunt; Autoinducers; Acetate; Phosphoenolpyruvate; Pyruvate

1. Introduction

Metabolic engineering can be defined as the modification of cellular enzymatic, transport and regulatory activities with the aim of strain improvement (Bailey, 1991). This discipline has been applied in *Escherichia coli* to improve productivity and yield in the synthesis of specific metabolites. Some of the strategies followed to achieve these goals include modification or elimination of the phosphoenolpyruvate (PEP):carbohydrate phosphotransferase system (PTS). This protein system belongs to the group translocator class of transporters, which are widespread in bacteria (Saier, 2002). One of

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the main functions of PTS is the transport and PEP-dependent phosphorylation of several sugars. This system is composed by the non-sugar-specific protein components Enzyme I and HPr whose function is to relay a phosphate group from PEP to sugar-specific IIA and IIB PTS proteins. PTS is also involved in the regulation of several cellular processes such as catabolite repression and chemotaxis (Postma et al., 1996). Therefore, PTS forms part of a global regulatory network that controls the capacity of cells to find, select, transport and metabolize several types of carbon sources.

Half of the PEP produced during glycolysis is consumed by PTS for glucose internalization. This metabolic constraint limits the amount of PEP available for the synthesis of several metabolites derived from this precursor when *E. coli* uses glucose as the carbon source. For this reason, considerable effort has been focused on developing *E. coli* strains that can transport glucose efficiently by a PEP-consumption independent mechanism. However, inactivation of PTS causes a wide range of effects due to its important role in the physiology of the cell. For example, a deletion of the *ptsHIcrr* operon in *E. coli* decreases glucose transport and growth rates (PTS^- phenotype) (Flores, 1995; Flores et al., 2002). As such, PTS^- strains are not suitable for production purposes. Therefore, further genetic modifications are required to increase glucose transport capacity in a PTS^- mutant. Different approaches have been reported to achieve this purpose with varying degrees of success (Flores et al., 1996; Chen et al., 1997; Chandran et al., 2003; Hernández-Montalvo et al., 2003).

We have obtained spontaneous mutants from PB11 (PTS^-) that lack the *ptsHIcrr* operon but grow faster on glucose than the PTS^- parental strain. These strains were isolated by an adaptive evolution process, in which PB11 was grown in a chemostat, with glucose fed at progressively higher rates (Flores, 1995; Flores et al., 1996). Initial characterization of these strains revealed that rapid glucose consumption and high growth rates depend on functional *galP* and *glk* genes that code for galactose permease (GalP) and glucokinase (Glk), respectively (Flores et al., 1996; Flores et al., 2002; Hernández et al., 2003). Using one of these mutants, PB12, we have shown that some of the PEP, which is not consumed for glucose transport due to the lack of PTS, could be redirected into the aromatic pathway, increasing the yield from glucose into the synthesis of 3-deoxy-D-*arabino*-heptulosonate-7-P (DAHP) (Gosset et al., 1996; Báez et al., 2001) and L-phenylalanine (Báez-Viveros et al., 2004). The carbon flux distribution in these isogenic PTS^+, PTS^- and PTS^-Glc^+ strains has been studied by biochemical analysis and nuclear magnetic resonance (NMR) spectroscopy. It was demonstrated that carbon flux distribution was modified at various nodes and portions of the central carbon metabolism in the PTS^- and PTS^-Glc^+ strains as compared to the wild-type JM101. This result clearly indicates that these PTS^- strains adjusted their metabolic capacities due to the absence of the PTS. For example, at the Embden–Meyerhof pathway (EMP), the carbon flux of the first node increased to 95% in the PB12 PTS^-Glc^+ (GalP/Glk) strain as compared to the wild-type JM101 (77%) and PB11 PTS^- (42%) parental strains (Flores et al., 2002). In agreement, it was also found that in PB12 the specific activities of Glk and Pgi increased approximately two- and four-fold, respectively, as compared with the wild-type strain JM101. As a result of these modifications, PB12 grows faster than PB11 (μ of 0.42 vs 0.1 h^{-1}) but slower than the wild-type strain (μ of 0.71 h^{-1}) (Flores et al., 2002).

It seems that during the process utilized for the isolation of PB12, at least two non-cotransducible mutations were selected to allow this strain to consume glucose in the absence of PTS at a much higher rate than PB11 (Flores, 1995; Flores et al., 1996). It has been reported that *E. coli* strains can adapt their metabolism for higher growth rates on specific carbon sources as a result of specific mutations (Raghunathan and Palsson, 2003). We are interested in identifying the mutations that were selected in PB12 and the genes involved in the differential utilization of glucose, carbon catabolism and other metabolic capacities present in these modified strains. In this contribution, we report a transcriptome analysis using RT–PCR measurements of more than 100 genes coding for enzymes that participate in the following pathways and processes: EMP, pentose pathway, TCA cycle, glyoxylate shunt, anaplerotic enzymes, gluconeogenesis, fermentation and respiration processes, and the *gal* operon. We have also determined the nucleotide sequences of 11 genes (regulatory and coding regions) from the three strains, looking for the mutations responsible for the observed changes.

2. Materials and methods

2.1. Bacterial strains, plasmids, media and growth conditions

E. coli strains used in this work are listed in Table 1. Strain PB12 was obtained from PB11, a PTS^- mutant derivative of *E. coli* JM101 (Flores, 1995; Flores et al., 1996). Duplicate cultures for RNA isolation and enzymatic assays were grown on 1 L fermentors on M9 medium with 2 g/l of glucose, at 37 °C, 600 r.p.m. and air flow rate of 1 v.v.m., starting at an OD_{600} of 0.05 and collected when growing in the log phase at an OD_{600} of 1.

For toluidine blue growth-sensitivity assays, cells were grown on peptone-agar plates containing 0.2 mg/ml of toluidine blue. Plasmid pBB31 that carries the wild-type