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CO-UTILIZATION OF GLUCOSE AND XYLOSE INCREASES GROWTH RATE WITHOUT AFFECTING PLASMID DNA YIELD OF ENGINEERED *E. coli*

EL CONSUMO SIMULTÁNEO DE GLUCOSA Y XILOSA INCREMENTA LA VELOCIDAD DE CRECIMIENTO SIN AFECTAR EL RENDIMIENTO DE ADN PLASMÍDICO EN UNA CEPA DE *E. coli* MODIFICADA GENÉTICAMENTE

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Abstract

Escherichia coli strains devoid of PTS system lack of catabolite repression allowing the simultaneous utilization of glucose and other carbohydrates. In this study, the use of xylose and glucose for the production of plasmid DNA (pDNA) was tested using the engineered strain *E. coli* PTS$^-$ GalP$^+$ Δ(*recA, deoR*). Growth rate and acetate production were similar using glucose or xylose as the carbon source, but pDNA yield ($Y_{P/x}$) was lower in cultures with xylose. However, when both carbon sources were co-utilized, growth rate increased by 39 %, and $Y_{P/x}$ recovered, consequently increasing the specific pDNA production rate by 28 %. Therefore, the used of glucose-xylose mixtures is an attractive alternative for pDNA production.

Keywords: *mixed sugars, metabolic engineering, pDNA vaccines, PTS.*

Resumen

Las cepas de *Escherichia coli* con el sistema de fosfotransferasa (PTS) inactivo carecen de represión catabólica, lo que permite el consumo simultáneo de glucosa y otros carbohidratos. En el presente estudio, se evaluó el uso de glucosa y xilosa en la producción de ADN plasmídico (ADNp) usando la cepa modificada *E. coli* PTS$^-$ GalP$^+$ Δ(*recA, deoR*). La velocidad de crecimiento y la producción de acetato fueron similares empleando glucosa o xilosa como única fuente de carbono, pero el rendimiento de ADNp ($Y_{P/x}$) fue menor en cultivos con xilosa. Sin embargo, cuando ambas fuentes de carbono fueron co-utilizadas, la velocidad de crecimiento se incrementó en 39 % y se recuperó $Y_{P/x}$, lo que en consecuencia incrementó en 28 % la velocidad específica de producción de ADNp. Por lo tanto, el uso de mezclas de glucosa-xilosa es una alternativa atractiva para la producción de ADNp.

Palabras clave: mezcla de carbohidratos, ingeniería metabólica, vacunas de ADN, PTS.

1 Introduction

Plasmid DNA (pDNA) is a promising alternative for the treatment and prevention of infectious diseases and several types of cancer (Lara *et al*., 2012). Progress in clinical trials implies that the need for pDNA production strains and processes will increase in the near future. Current efforts to increase pDNA productivity have focused on improving production strains (Borja *et al*., 2012; Gonçalves *et al*., 2013; Pablos *et al*, 2012), vector (Mairhofer *et al*., 2010), induction methods (Silva *et al*., 2011; Jaén *et al*., 2013) and cultivation media (Voss *et al*., 2004). The most usual carbon source (C-source) used for pDNA production is glucose. However, fast aerobic growth on glucose invariably triggers acetate production, known as overflow metabolism. Acetate accumulation in the broth is highly undesirable (Lara, 2011). We have previously shown that overflow metabolism is effectively prevented by substituting the natural glucose transport system (phosphotransferase system, PTS) by the galactose permease (GalP) (De Anda *et al*., 2006). Further genetic modifications have been applied to develop strains with higher pDNA yields and low aerobic acetate production by overflow metabolism, leading to the production of more than 180 mg/L of a pDNA experimental vaccine in batch mode using 100 g/L of initial glucose concentration (Borja *et al*., 2012).

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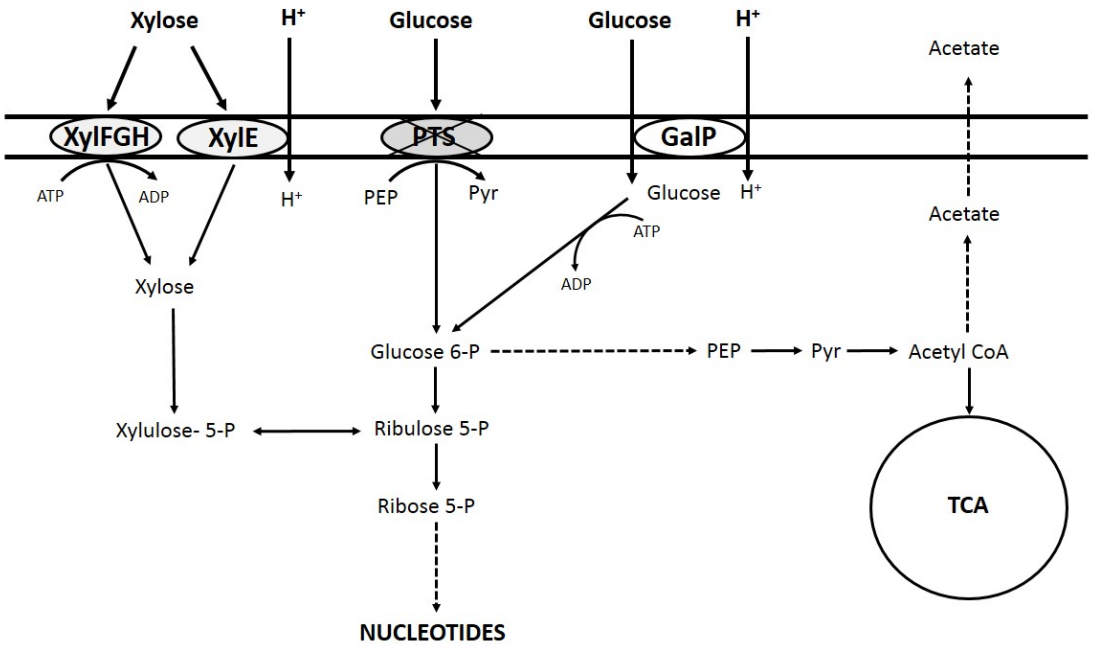

Fig. 1. Simplified metabolic pathways involving co-utilization of glucose and xylose by the engineered *E. coli* strain. Glucose is transported through the galactose permease (GalP) instead of the phosphoenolpyruvate:phosphotransferse system (PTS). Xylose can be transported through different systems, mainly XylFGH and to a lesser extent XylE (Cirino *et al*., 2006). PEP: phosphoenolpyruvate; Pyr: pyruvate; TCA: tricarboxylic acid cycle.

Besides the low acetate production, a relevant feature of PTS mutants is that they lack catabolite repression by glucose, which makes possible the simultaneous utilization of glucose and other C-sources. For instance, the co-utilization of glucose and glycerol by PTS- *E. coli* strains resulted in an increased production of aromatic compounds (Martínez *et al*., 2008). In the case of biofuels (Eiteman *et al*., 2008; Chiang *et al*., 2013) and xylitol (Cirino *et al*., 2006) production by *E. coli*, the simultaneous utilization of glucose and xylose is of particular interest. However, the use of carbon mixtures has not been evaluated, to the best of our knowledge, for pDNA production. In this study, the use of glucose and xylose mixtures by an *E. coli* strain for the production of a pDNA vaccine was evaluated. Xylose was selected as an additional C-source since it is incorporated to the pentose phosphate pathway, which is closely involved in the catabolism of nucleotides (see Fig. 1). Therefore, it may be expected that glucose could be mainly used for energy and biomass generation, while xylose could be utilized for the synthesis nucleotide building blocks.

2 Materials and methods

2.1 Bacterial strain, plasmid model and pre-cultures

The engineered strain used in this study was a W3110 PTS$^-$ GalP$^+$ Δ(*recA deoR*) mutant. The strain was transformed with an experimental pDNA vaccine against mumps, denoted here as pHN. Plasmid pHN was constructed from the pcDNA3.1(+) plasmid (Invitrogen), which contains the pUC origin of replication and an ampicillin resistance gene. A viral haemagglutinin-neuraminidase gene was cloned under transcriptional control of the cytomegalovirus promoter (Herrera *et al*., 2010). 1.5 mL of cryopreserved (at 80 °C in 40 % glycerol) cells were grown in 50 mL of mineral medium (Soto *et al*., 2011) added with 5 g/L of the C-source to be used in the main culture and 0.1 g/L of ampicillin disodium salt. Cells were cultured for 14 h at 37 °C, washed in sterile 0.9 % NaCl and subsequently used to inoculate the main cultures.

2.2 Shake flask cultures

The main cultures were carried out in 250 mL baffled Erlenmeyer flasks containing 50 mL of the mineral medium plus the C-source and ampicillin disodium salt (0.1 g/L).

When used as the only C-source, initial concentration of glucose or xylose was 5 g/L. When used in mixture, the concentrations were 2.5 and 7 g/L for glucose and xylose, respectively. The shake flasks were maintained in an orbital shaker at 250 rpm and 37 °C of temperature.

2.3 Analytical methods

Cell growth was followed by absorbance at 600 nm and transformed to cell dry weight by using a predetermined factor (0.38 g_X/OD). pDNA was extracted from 2 mg of wet biomass samples taken at the middle of the exponential growth phase, using a commercial kit (Qiagen Spin Mini Prep kit, Hilden, Germany), following the instructions of the manufacturer and quantified spectrophotometrically at 260 nm using a Nanodrop UV spectrophotometer ND-2000 (NanoDrop, Wilmington, DE). Supercoiled pDNA fraction was determined by agarose gel electrophoresis (Soto *et al*., 2011). Glucose was measured by using the YSI 2900 Biochemistry Analyzer (Yellow Springs Instruments, Yellow Springs, OH), whereas xylose and acetate were measured by HPLC using an anionic interchange column (Soto *et al*., 2011). Acetate was measured at the time point of carbon source(s) exhaustion.

2.4 Calculation methods

The reported values of biomass yields on substrate ($Y_{X/S}$) were calculated from the slope of the biomass vs. substrate concentration plot during the exponential growth phase. The specific rates were calculated with the proper mass balances over the time periods involved (exponential growth phase). The average values were tested for statistical differences (Anova) using a commercial software (SigmaPlot, Systat Software, San Jose, CA).

3 Results and discussion

3.1 Cultures using a single C-source

The growth profile of the engineered strain using glucose or xylose as the only C-source is shown in Fig. 2, whereas the main kinetic and stoichiometric parameters can be seen in Table 1. Exponential growth was observed for both strains from the first hour of cultivation and the specific growth rate (μ) during exponential growth phase were similar for both C-sources, which is in agreement with results of other authors (Xia *et al*., 2012). The biomass yield ($Y_{x/s}$) was also lower using xylose compared to glucose (about 30 or 16 % lower on g or mmol of C-source basis, respectively). In consequence, the specific substrate uptake rate was considerably higher for xylose (q_{xylose}) than for glucose ($q_{glucose}$) (70 % or 100 % higher on g or mmol of C-source basis, respectively). Acetate production was also similar when glucose or xylose was used. The specific pDNA yield ($Y_{P/x}$) was 17 % lower in cultivations using xylose, compared to those using glucose as carbon source, and the supercoiled pDNA fraction was similar in both cases. Overall, these results indicate that glucose is a better C-source for both, pDNA and biomass synthesis than xylose using the engineered strain.

3.2 Cultures using a glucose-xylose mixture

A mixture of glucose and xylose as C-sources was used to evaluate the effects on pDNA production. Considering the difference in uptake rates, xylose should be in higher concentrations than glucose if the effect of the co-utilization of both C-sources is to be evaluated during a longer growth period. Therefore, it was decided to use 7 g/L of xylose and 3 g/L of glucose. The growth and C-sources consumption are shown in Fig. 3, and the stoichiometric and kinetic parameters are included in Table 1. As seen in Fig. 3, the engineered strain grew exponentially from the inoculation, consuming both C-sources simultaneously, as expected from its genotype.

Table 1. Kinetic and stoichiometric parameters of the engineered *E. coli* strain growing in the different C-sources. All the values were obtained during exponential growth phase except were indicated.

Parameter (units)	Glucose	Xylose	Glucose-Xylose
$\mu(h^{-1})$	0.29 ± 0.01	0.28 ± 0.01	0.39 ± 0.02
Acetate (g/L)*	0.01± 0.01	0.03 ± 0.01	0.09 ± 0.02
$Y_{P/x}$ (mg/g)	3.46 ± 0.19	2.81 ± 0.33	3.26 ± 0.12
q_p (mg/g h)	1.00 ± 0.05	0.79 ± 0.06	1.28 ± 0.06
$(Y_{x/s})_{glu}$ (g/g)	0.40 ± 0.04	N. A.	0.74 ± 0.03
$(Y_{x/s})_{xyl}$ (g/g)	N. A.	0.28 ± 0.01	0.52 ± 0.03
$(Y_{x/s})_{overall}$ (g/gC)	1.20 ± 0.08	0.81 ± 0.02	0.70 ± 0.12
q_{glu} (g/g h)	0.62 ± 0.06	N. A.	0.54 ± 0.02
q_{xyl} (g/g h)	N. A.	1.05 ± 0.02	0.78 ± 0.04
Supercoiled fraction	77 ± 5 %	78 ± 7 %	80 ± 3 %

*Determined at the point of C-source exhaustion

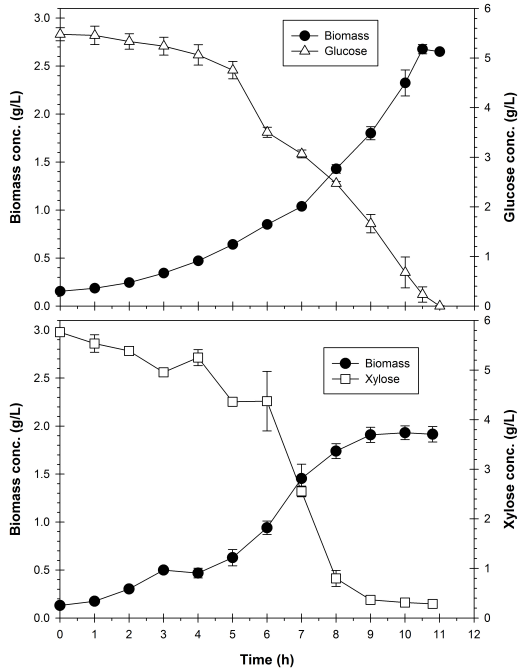

Fig. 2. Growth profile of the engineered *E. coli* strain growing in glucose (top panel) or xylose (bottom panel) as the only C-source. Error bars show the difference between duplicate experiments.

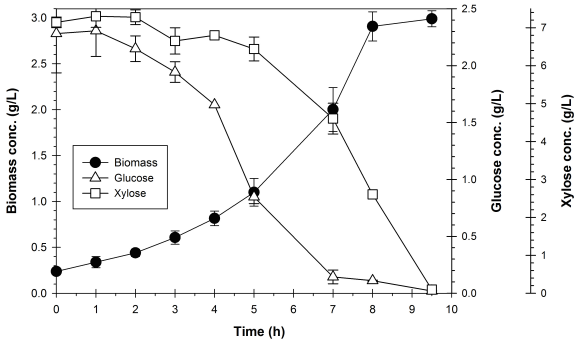

Fig. 3. Growth profile of the engineered *E. coli* strain growing in a mixture of glucose and xylose as C-sources. Error bars show the difference between duplicate experiments.

Moreover, both C-sources were depleted at the same time. The simultaneous consumption of xylose and glucose resulted in a 39 % of μ, compared to the growth on a single C-source (Table 1). Although acetate accumulated to higher values compared to cultivations with a single C-source, the g of acetate produced per g of C-source(s) is was very similar. The biomass yields calculated for each single C-source in the mixture are higher than in cultivations with a sole C-source. Nevertheless these values represent the simultaneous consumption of both C-sources, and therefore cannot be regarded as stoichiometrically true.

Despite the increase in μ, the specific C-source uptake rates during co-utilization were lower than the obtained in single C-source cultivations. The $Y_{p/x}$ was higher using xylose-glucose mixtures than using only xylose, and very similar to the obtained using only glucose (Table 1). Moreover, due to the increase of μ, the specific pDNA production rate (q_p) was ca. 28 % higher than the value obtained with glucose, whereas the supercoiled fraction was similar to the obtained in cultivations with a single carbon source.

The results obtained with the use of xylose and glucose suggest that the co-utilization of substrates helps to partially alleviate the metabolic burden imposed by the plasmid replication (Roskov *et al*., 2004, Silva *et al*., 2011). This applies to the data shown in Table 1, since the values were obtained when both C-sources were consumed simultaneously. Although the fluxes distribution using both C-sources simultaneously is not studied here, it could supposed that xylose is effectively incorporated into the pentose phosphate pathway (PPP) for biomass and ribose synthesis, whereas glucose could be used mainly as energy source for synthetic activities. This is well in agreement with previous results showing that plasmid replication increases ATP demand (Wang *et al*., 2006) and that the metabolic state of maximum pDNA productivity of an *E. coli* PTS- GalP+ mutant results in a higher carbon flux to the PPP (Wunderlich *et al*., 2014). In fact, it has been demonstrated that over expression of PPP genes reduces metabolic burden (Flores *et al*., 2004) and increase pDNA yields (Wang *et al*., 2006).

Conclusions

While glucose is a better C-source for biomass and pDNA synthesis by the engineered *E. coli* strain, the use of a glucose-xylose mixture restored growth rate and pDNA yields, while displaying a very low overflow metabolism. All together, the results obtained using xylose-glucose mixtures show that this is a simple, yet effective strategy to increase pDNA productivity by engineered *E. coli* strains. This is, to the best of our knowledge, the first report on the use of C-sources mixtures for improving pDNA production by *E. coli*.

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