Engineering *E. coli* to increase plasmid DNA production in high cell-density cultivations in batch mode

Gheorghe Manuel Borja[1], Guillermo Gosset[2], Octavio T. Ramírez[1], Alvaro R. Lara[3]
Departamentos de [1]Medicina Molecular y Bioprocesos e [2]Ingeniería Celular y Biocatálisis, IBTUNAM
y de [3]Procesos y Tecnología, UAM-Cuajimalpa
E-mail: gheorghe@ibt.unam.mx
Plasmid DNA, DNA vaccines, Overflow metabolism, E. coli, Batch cultivation, Acetate

Introduction.

Recent advances in therapies using plasmid DNA (pDNA) imply that better strains for its production will be needed in a near future. pDNA is produced in high cell-density cultivations (HCDC) of *Escherichia coli.* The typical limitations in practical cultivations of *E. coli*, like aerobic acetate production, remain as serious challenges for the fast and effective pDNA production. We have previously demonstrated that the substitution of the natural glucose transport system (PTS) by the over-expressed galactose permease in *E. coli* allows efficient growth while strongly decreases acetate production. The modified strain, named VH33, has been tested for pDNA production using high initial glucose concentrations in order to reach high cell densities in batch mode (1). Notwithstanding the higher production of such strain, compared to its wild type (W3110), its production levels remain low if compared to commercial strains like DH5α. In the present work, several genetic modifications were made to VH33 to further improve pDNA production

Methods.

The kinetic and stoichiometric effects of the mutations were evaluated in shake flasks during the production of a 6 kb plasmid bearing an antigen gene against mumps. The best producer strain was cultivated in a lab-scale bioreactor using 100 g/L of glucose to achieve HCDC in batch mode and compared with DH5α under the same conditions. Plasmid supercoiling degree was also evaluated. The *recA* (to reduce pDNA segregation and deregulate pDNA replication), *deoR* (to deregulate nucleotide synthesis) and *nupG* (to avoid nucleotide export) genes were inactivated independently and in combination from strain VH33 using the protocol described elsewhere (2).

Results.

The different mutations had effects in the specific growth rate, glucose uptake rate and pDNA yields at different levels and will be shown during the presentation of the present work. Whereas the pDNA yield ($Y_{P/X}$) of VH33 was 1.16 mg/g, the triple mutant VH33 Δ *recA deoR nupG* reached a yield of 4.22 mg/g, while the acetate production remained very low. When cultivated using a high glucose concentration, the engineered strain produced 182 mg/L of pDNA, 40 g/L of biomass and only 2 g/L of acetate. In contrast, DH5α produced only 70 mg/L pDNA and accumulated 9 g/L of acetate. Furthermore, the supercoiling degree of the pDNA produced by the triple mutant was higher than 80 % (figure 1). Such pDNA concentration is, to the best of our knowledge, the highest ever reported for a batch cultivation of *E. coli* (3-5).

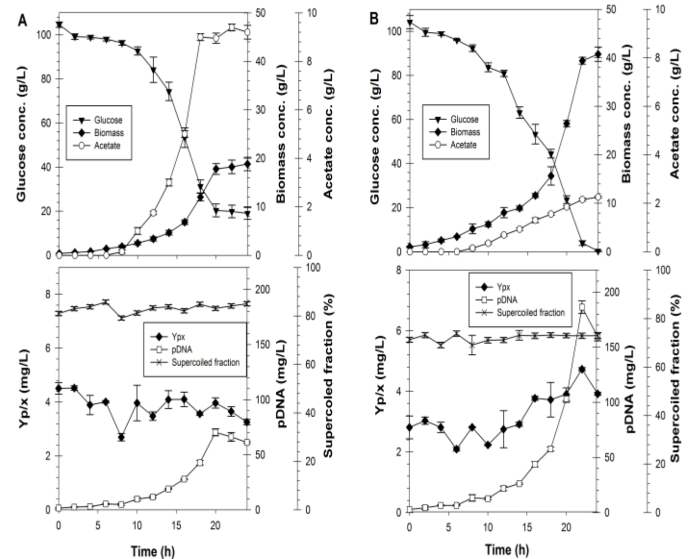

Fig.1 Growth profile of strains DH5α (A) and VH33 Δ (*recA deoR nupG*) In bach cultivations an initial glucose concentration of 100 g/L. Top panels: glucose, biomass and acetate concentrations. Bottom panels: pDNA yield on biomass, pDNA concentration and pDNA supercoiled fraction. Error bars show the standar deviation between duplicates

Conclusions. The strain developed in the present work and its cultivation using elevated glucose concentrations represent an attractive technology for fast and efficient pDNA production and a valuable alternative to fed-batch cultivations of commercial strains.

Acknowledgements.

This work was supported by CONACyT grants 84447 and 126663, PROMEP 47410089 and DGAPA-UNAM IN203212.

References.

1) Soto R, Caspeta L, Barrón BL, Gosset G, Ramírez OT, Lara AR. 2011. High cell-density cultivation in batch mode for plasmid DNA vaccine production by a metabolically engineered *E. coli* strain with minimized overflow metabolism. Biochem Eng J. 56(3): 165-171.
2) Datsenko KA, Wanner BL. 2000. One-step inactivation of chromosomal genes in Escherichia coli K-12 using PCR products. Proc Natl Acad Sci USA.97(12):6640-5.
3) Wang Z, Le GW, Shi YH, Wegrzyn G: Medium design for plasmid DNA production based on stoichiometric model. Proc Biochem 2001, 2001 (36):1085–1093.
4) Duttweiler HM, Gross DS: Bacterial growth medium that significantly increases the yield of recombinant plasmid. Biotechniques 1998, 24 (3):438–444.
5) Zheng S, Friehs K, He N, Deng X, Li Q, He Z, Xu C, Lu Y: Optimization of medium components for plasmid production by recombinant E. coli DH5α pUK21CMVβ1.2. Biotechnol Bioproc Eng 2007, 12(3):213–221.