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ENHANCED PLASMID DNA PRODUCTION IN SHAKE FLASK BY ENZYME-CONTROLLED GLUCOSE RELEASE AND ENGINEERED ESCHERICHIA COLI

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Introducction. Shake flasks are the most widely used biorreactor in early stages of product and process development. However, intrinsic limitations like low mass transfer result in low cell densities and product formation. This problem is of particular relevance in the case of plasmid DNA (pDNA) vaccines, since relatively high amounts of pDNA are needed to evaluate the drug candidates. Therefore, better option for increasing the cell density, pDNA yield on biomass, or both in shake flask are needed. In this work, we evaluate the use of a prototype rich medium with enzyme-controlled glucose release and an engineered *E. coli* strain as a strategy to improve pDNA production in shake flasks. This system mimics the conditions of a fed-batch culture. Three different amounts of enzyme were tested. The use of the prototype medium and engineered *E. coli* resulted in an increase of up to 200 % of pDNA productivity, compared to the production using a commercial strain under the same conditions.

Methodology. The Biosilta Enpresso B Plasmid prototype (EBPP) medium was used according to the manufacturer and using 3 different amounts of enzyme for glucose release: 1, 2 and 3 U/L. The commercial DH5a strain and the engineered strain VH33 Δ(*recA deoR*) were cultured at 39 °C. The engineered strain has been shown to yield good amounts of pDNA while producing very low amounts of acetate by overflow metabolism. Both strains were tested for the production of a 6.0 Kbp pDNA. Samples were take regularly, and biomass was followed as cell density pDNA was purified using commercial kits based on alkaline lysis tabolites were quantified by HPLC. pDNA concentration was measured spectrophotometrically and the supercoiled fraction determines by agarose gels electrophoresis.

Results. Three enzyme concentrations for releasing glucose were used: 1, 2 and 3 U/L. While both strains reached similar cell densities ranging from 15 to 25 units of OD600nm, the engineered strain produced biomass faster than DH5α. The higher pDNA yields on biomass for both strains were obtained when 3 U/L of enzyme were used. Under these conditions, 34.7 ± 2.5 mg/g of pDNA were produced by DH5α after 24 h of culture. In contrast, under the same conditions, the engineered strain produced 65.7 ± 0.5 mg/L of pDNA after 20 h of culture, which represents an increase of 89 and 200 % on pDNA concentration and productivity, respectively. pDNA supercoiled fraction was higher than 90 % for the commercial strain and closet o 80 % for the engineered one.
Organic acids were detected in the samples, indicating oxygen limitation. Nevertheless, the engineered strain accumulated less organic acids under all conditions, which also resulted in pH values closer to neutrality than cultures of the commercial strain.

Conclusions. The use of the EBPP medium with 3 U/L of enzyme substantially increased the YpDNA/X, compared to batch cultures using a mineral medium for the production of the same plasmid. Under these conditions, the amount of pDNA produced by the engineered strain was nearly the double than the produced by the strain DH5α. The accumulation of organic acids, although lower for the engineered strain, evidences that some oxygen limitation occurs by the end of the culture. Overall, the combination of the engineered strain and the EBPP medium with slow glucose release, is a valuable option for pDNA production in shake flasks.

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