TAQ POLYMERASE GENE EXPRESSION UNDER AN OSMORREGULATED PROMOTER

Yeosvany Cabrera[1], Duniesky Martinez[1], Enrique R. Pérez[1], Gabriel J. Márquez[2], Manuel de J. Luis[1].

[1] Centro de Ingeniería Genética Biotecnología , Sancti Spíritus,60200, Cuba Fax: (53-41) 28489. [2] Centro de Ingeniería Genética Biotecnología , La Habana,6162, Cuba ycabrera@cigbss.ssp.sld.cu

Palabras clave: Taq polymerase, osmoregulation, *ProU*

Introduction: Expression of the proU operon of Escherichia coli is directly proportional to the osmolarity of the growth medium; pOSEX family vectors are plasmids containing the proU promoter and other regulator regions (1). The expression level of Taq Pol I (PCR enzyme) in the native host is very low (0.01-0.02% of total protein). Cloning of the Taq Pol I gene and its expression in E.coli have been reported to increase expression level (2).

We describe the cloning and expression of Taq pol I gene under ProU promoter and the effects of different culture media and/or inductor concentration in the specific growth rate of E.coli MKH13 (pOTPEX) and in Taq pol I enzyme levels. The pOTPEX plasmid for the expression of Taq pol I, it is very advantageous to make a regulated, non-toxic, simple and cost-effective manner induction using just NaCl.

Methodology: EcoRV- SalI fragment was excised from the plasmid pTQ22 carrying the total coding Taq pol sequence (3) and inserted into the pOSEX4 expression vector. As a result, the Taq pol I gene was located under the control of the ProU promoter. Recombinant plasmid, pOTPEX, was transformed into E. coli MKH13 (1). The production of Taq pol I by transformed E.coli was detected by ^{35}S dATP incorporation and PCR to heat-treated extracts.

To evaluate the effects of media on cell density and gene expression, we tried three media; I (LB medium without NaCl), II M9 medium containing Glc 4 g/L, and III medium II with Yeast Extract 5 g/L. The induction of gene expression was achieved by adding of NaCl at different final concentration at the beginning of the culture. All the media contained Ampicillin 100µg/mL. Culture condition: 300 ml bacterial cultures, 37 ^{o}C and 250 rpm.

Results and Discussion: Gene expression by ^{35}S dATP incorporation into medium I resulted in an enzyme activity of 85 000 Units per gram of biomass, according to total count rate. PCR results indicated that the Taq Pol I enzyme produced was active and suitable.

The higher growth was obtained in media III and I in 8 hours of non-induced culture. The lesser growth in medium II could be caused by limited nutrient concentration, and accumulation of organic acids in the culture.

When inducing the system with NaCl at 250mM concentration, a decrease of all specific growth rates was observed, caused by osmotic stress; strain MKH13 is particularly more sensitive to osmotic up shock. Activity was assayed in media III and I by comparing the total activity in different phases of growth and we obtained a maximum value in medium I at the end exponential phase. However, it was lower at the end of the stationary phase, perhaps due to cell physiological changes caused by nutrient limitation and not by osmotic influence; similar results were obtained in medium III, but the total activity was lower than in medium I. We therefore selected medium I for further study. The polymerase activity values obtained for media in non-induced conditions indicate an osmolarity sufficient for basal activity or an inefficient promoter regulation at transcriptional level. The influence of different NaCl concentrations on total enzyme activity in the same growth phase was analyzed in medium I. The growth rate decreased as osmolarity of the medium increased due to an increment in NaCl concentration. Expression of proU operon in MKH13 is directly proportional to osmolarity of the growth medium for 175 mM and 250 mM NaCl concentrations but, in the case of 300 mM and 340 mM, we obtained an unexpected reduction of activity, in contradiction with previous reports (4). This decrease could explain the low activity values in induced medium III compared to those of induced medium I, since the osmolarity in medium III is higher than the medium I osmolarity with the same NaCl concentration.

Conclusions: NaCl 250 mM make regulated, non toxic and cost-effective induction of Taq pol I gene under proU promoter in MKH13 in medium I

Acknowledgment: We are grateful to Dr. Prof. Edhard Bremer from the Biology Department of Marburg University, Germany for providing the *Escherichia coli* MKH13 strain, plasmid pOSEX4 and all his helpful advice.

References:

1. Herbst, B., Kneip, S., Bremer, E., 1994. pOSEX: vectors for osmoticaly controlled and finely tuned gene expression in *Escherichia coli*. *Gene*. 151: 137-142.

2. Engelke, D.R., Krikos, A., Bruck, M.E., Ginsburg, D., 1990. Purification of *Thermus aquaticus* DNA polymerase expressed in *Escherichia coli*. *Anal. Biochem*. 191:396-400.

3.Leonart, R, Campos, M., Suárez, A., Vázquez, R., de la Fuente J., Herrera, L., 1991. Molecular Cloning of the gene; expression in *E.coli* and purification of the *Thermus aquatic* DNA Polymerase I. *Acta Biotechnol*. 11:67-71.

4. Luncht, J.M. & Bremer, E.,1994. Adaptation of *Escherichia coli* to high osmolarity environments: Osmoregulaton of the high affinity glycine betaine transport system ProU. *FEMS Microb*. Rev. 14:3-20.