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Osmoregulated TAQ polymerase gene expression in *Escherichia coli*

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ABSTRACT. The *Thermus aquaticus* DNA Polymerase I (Taq Pol I) gene was cloned into the pOSEX4 plasmid under the osmo-inducible promoter proU and subsequently expressed into the *Escherichia coli* MKH13 strain. The suitability of the enzyme in polymerase assays was determined in standard 35S dATP incorporation tests and by PCR. The Taq Pol I expression in this system, which is under the control of the osmotic pressure in the growth medium, was analyzed in different media and in different sodium chloride concentrations. A study of the osmolarity effects in the growth of the strain and in Taq Pol I expression shows that an increase in sodium chloride concentration limits the growth. At 0.25 M of NaCl maximum activity was observed; at higher values of osmolarity, we found an unexpected decline of activity. This is the first report of using the pOSEX vector for the expression of an heterologous protein and it is very advantageous to make a regulated, non toxic, simple and cost-effective manner of induction in a biotechnology process using just NaCl or other non-permeable osmolyte.

Key words: Taq pol I, MKH13 *E. coli*, gene expression.

INTRODUCTION

The osmotic strength of the environment is an important physical parameter that influences the ability of organisms to grow. Osmoadaptation in bacteria entails a two-step process. First, high intracellular concentrations of K⁺-glutamate accumulate due to increased K⁺ uptake and concomitant de novo synthesis of counterions such as glutamate. Second osmoprotective compounds such as trehalose, proline, glycine betaine and proline betaine accumulate by either synthesis or uptake from the environment. Accumulation of these osmoprotectans then triggers an efflux of K⁺ from the cytoplasm. Through out this series of events, turgor is restored, and the cell can resume growth in a high osmolarity environment.^{1,14}

The ProU system from *E. coli* and *S. typhimurium* has a very high affinity with glycine betaine and is a multi-component, binding-protein-dependent transport system.^{2,3} Ex-

RESUMEN. El gen que codifica para la ADN polimerasa I de *Thermus aquaticus* (Taq Pol I) se clonó en el vector plasmídico pOSEX4 bajo el control del promotor osmoinducible proU y consecuentemente expresado en la cepa *Escherichia coli* MKH13. La actividad de la enzima fue determinada por la cuantificación de 35S dATP incorporado y por PCR. La expresión de la Taq pol I en este sistema, la cual está bajo el control de la presión osmótica del medio de crecimiento, se analizó en diferentes medios y concentraciones de cloruro de sodio, utilizado como inductor. Un estudio de los efectos de la osmolaridad en el crecimiento y en los niveles de expresión de Taq pol muestra que un incremento en la concentración de cloruro de sodio limita el crecimiento. Se observó una actividad máxima a 0.25 M de NaCl, a valores mayores de osmolaridad se encontró un inesperado decrecimiento de la actividad. Este es el primer reporte sobre el uso del vector pOSEX para la expresión de proteínas heterólogas, siendo muy ventajoso para una forma de inducción regulada, no tóxica y económicamente factible en procesos biotecnológicos con el solo uso de NaCl u otro osmolito no permeable.

Palabras clave: Taq pol I, *E. coli* MKH13, expresión génica.

pression of this 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.⁶ Osmotically regulated expression of the proU operon can be triggered in both minimal and rich media by a variety of ionic (e.g. NaCl) or non-ionic (e.g. sucrose) osmolytes.

The thermostable DNA polymerase I (Taq pol I) from *Thermus aquaticus* greatly improves the yield, specificity, automation, and usefulness of the polymerase chain reaction (PCR) method of amplifying DNA fragments.^{11,12} The expression level of Taq Pol I in the native host is very low (0.01-0.02% of total protein).⁴ Cloning of the Taq Pol I gene and its expression in *E. coli* have been reported to increase expression level.^{5,7,8}

In this paper, we describe the cloning and expression of Taq pol I gene under an osmoinducible promoter of vector pOSEX4 and the effects of different culture media and/or inductor concentration in the growth of *E. coli* MKH13 (pOTPEX) and in Taq pol I enzyme levels. The pOSEX vector for the expression of a heterologous protein, Taq pol I, it is very advantageous to make a regulated, non toxic, simple and cost-effective manner induction using just NaCl.

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MATERIAL AND METHODS

Bacterial strain and plasmid

- *E. coli* MKH13 (Δ (*putA*) 101 Δ (*proP*) 2 Δ (*proU*) 608)]* derivative of the *E. coli* K-12 strain MC4100 was used as the cloning host for the Taq polymerase I gene expression.
- The plasmid pOSEX4⁶ was used as expression vector of proteins in *E. coli* under the control of the osmo-inducible *proU* promoter. Ampr.
- pTQ22,⁸ a pUC19 carrying the DNA polymerase gene from *Thermus aquaticus* and Ampr.

Reagents

Restriction endonucleases, T4 DNA ligase (New England Biolabs and Heber Biotec S.A.) were used according to standard procedure. Crude salmon sperm DNA (Sigma) was activated by incubation at 40°C for 24 h at 1.3 mg/ml in 10 mM Tris-HCl (pH7.2), 5 mM MgCl₂,⁹ α^{35S} dATP was purchased from Amersham.

Medium composition

Medium I was LBON, Luria Bertani (medium) without NaCl; medium II was based on an M9 medium containing KH₂PO₄ 3 g, Na₂HPO₄ 6 g, NaCl 0.5 g, NH₄Cl 7 g, MgSO₄ 1M 2 ml, CaCl₂ 1M 0.1 ml and Glucose 4 g per liter; medium III was medium II with additional Yeast Extract 5 g/l. The induction of gene expression was achieved by adding of NaCl at 250 mM as final concentration in the growth media at the beginning of the culture. All the media contained Ampicillin 100 μ g/ml.

Culture condition

Cultures were performed in a 1 liter Erlenmeyer flask containing 250 ml of medium at 37°C and 250 rpm. Evaluation of gene expression was carried out in medium I with and without NaCl. To determine the effects of the media on the growth and the gene expression, inducing and non-inducing media were used in the same culture condition. Several final concentrations of NaCl (175 mM, 250 mM, 300 mM, 340 mM) were employed in medium I to evaluate the influence of osmolality. Broth samples from the induced and non-induced cultures were collected immediately after inoculation and at regular intervals of 1 hour there-

after. Cell density was measured as OD at 660 nm, either directly or after dilution of the culture samples.

Enzyme activity assay

Previous activity assays crude extracts were heated at 70°C during one hour. DNA polymerase assays were performed in a 20 μ l volume at 70°C. Assay conditions were 10 mM Tris (hydroxymethyl) aminomethane HCl (pH 9 at 25°C), 50 mM KCl, 0.1% Triton X-100, 25 mM MgCl₂, 250 mM each of the dNTP, 7 μ Ci (α^{35S} dATP (600 Ci/mmol), 0.6 mM of activated salmon sperm DNA template. The α^{35S} dATP incorporated was measured in a LKB Pharmacia scintillation spectrometer. One unit is defined as the amount of enzyme required to catalyze the incorporation of 10 nmoles of dNTP into an acid-insoluble form in 30 minutes at 70°C under these conditions.

Activity of crude extracts in PCR

Titration of enzyme fractions using polymerase chain reaction amplification from lambda phage genomic DNA was performed in 50 μ l of the reaction with 1 μ l of the diluted heat-treated extract samples added just before overlaying with mineral oil and beginning temperature cycling. The reaction contained 10 mM Tris (hydroxymethyl) aminomethane HCl (pH 9 at 25°C), 50 mM KCl, 0.1% Triton X-100, 25 mM MgCl₂, 0.15 mM each of dNTP, 0.5 μ g of template DNA and 25 pmoles of each of the two 25 mer primers (5'GATGAGGTTTCGTGTCCGTACAACACTGG3') and (5'CTGCGATATCGCGGATGAAGCAACG3') that amplified a 962 base pairs fragment of the phage lambda genome in standard PCR amplifications. Aliquots of 5 μ l were analyzed by electrophoresis through agarose gels.

RESULTS AND DISCUSSION

Construction of pOTPEX recombinant plasmid

EcoRV- Sall fragment was excised from the plasmid pTQ22 carrying the total coding Taq pol sequence 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 confirmed by restriction enzyme digestion profiles, and used to transform *E. coli* MKH13. The production of Taq DNA polymerase by transformed *E. coli* was detected by ^{35S} dATP incorporation and PCR to heat-treated extracts.

Gene expression by ^{35S} dATP incorporation into medium I resulted in an enzyme activity of 85,000 Units per gram of wet weight, achieving levels of expression around 1% of the total cell protein, similar to Taq pol expression

*M. Haart and E. Bremer, unpublished results

levels in *E. coli* reported by Leonard et al., and Lawyer et al., but greatly minor to those reached by Engelke et al., Results shown in Fig. 1 indicate that the Taq Pol I enzyme produced by MKH13 (pOTPEX) was active and suitable for PCR.

The effects of media on growth and gene expression

To evaluate the effects of media on cell density and gene expression, we tried three kinds of media; one with low osmolarity (I) and two with high osmolarity (II, III). In general, the cultures exhibited an exponential phase followed by a stationary phase. The higher growth was obtained in media I and III in 8 hours of non-induced culture (Fig. 2). The lesser growth in medium II could be caused by limited nutrient concentration, and accumulation of organic acids in the culture under aerobic conditions using glucose as unique carbon source which lowers the optimum pH for growth.

When inducing the system with NaCl at 250 mM concentration, a growth decreasing was observed in the three media assayed, caused by osmotic stress; strain MKH13 is particularly more sensitive to osmotic upshock because it carries mutations which render it entirely deficient in glycine betaine uptake and synthesis, one of the most important osmoprotectants in *E. coli*. Additionally, the recombinant product could influence in the growth and metabolic activity of the host, especially after induction, however it is not the main cause of growth decreasing with our expression levels (Fig. 2).

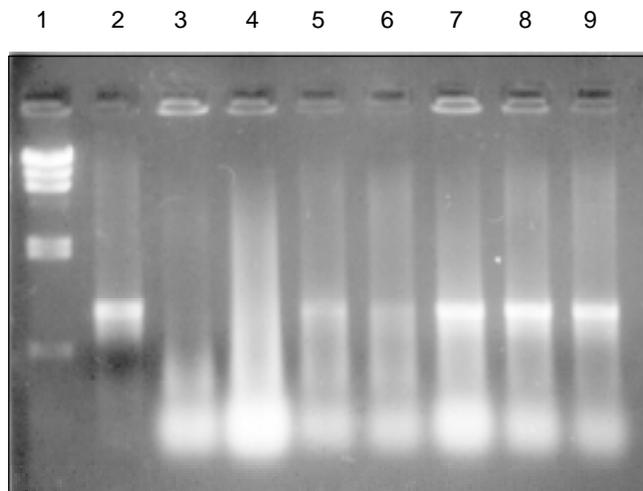


Figure 1. PCR amplification of a 962 base pairs fragment of lambda phage DNA using heat-treated extracts from a culture of MKH13 (pOTPEX) strain in medium I. Lane 1 molecular weight marker (lambda DNA HindIII digested), lane 2 positive control, lane 3 extract dilution from a non-induced culture, lanes 4-6 extract dilutions from induced culture with NaCl at 300 mM, lanes 7-9 extract dilutions from a culture with NaCl at 250 mM.

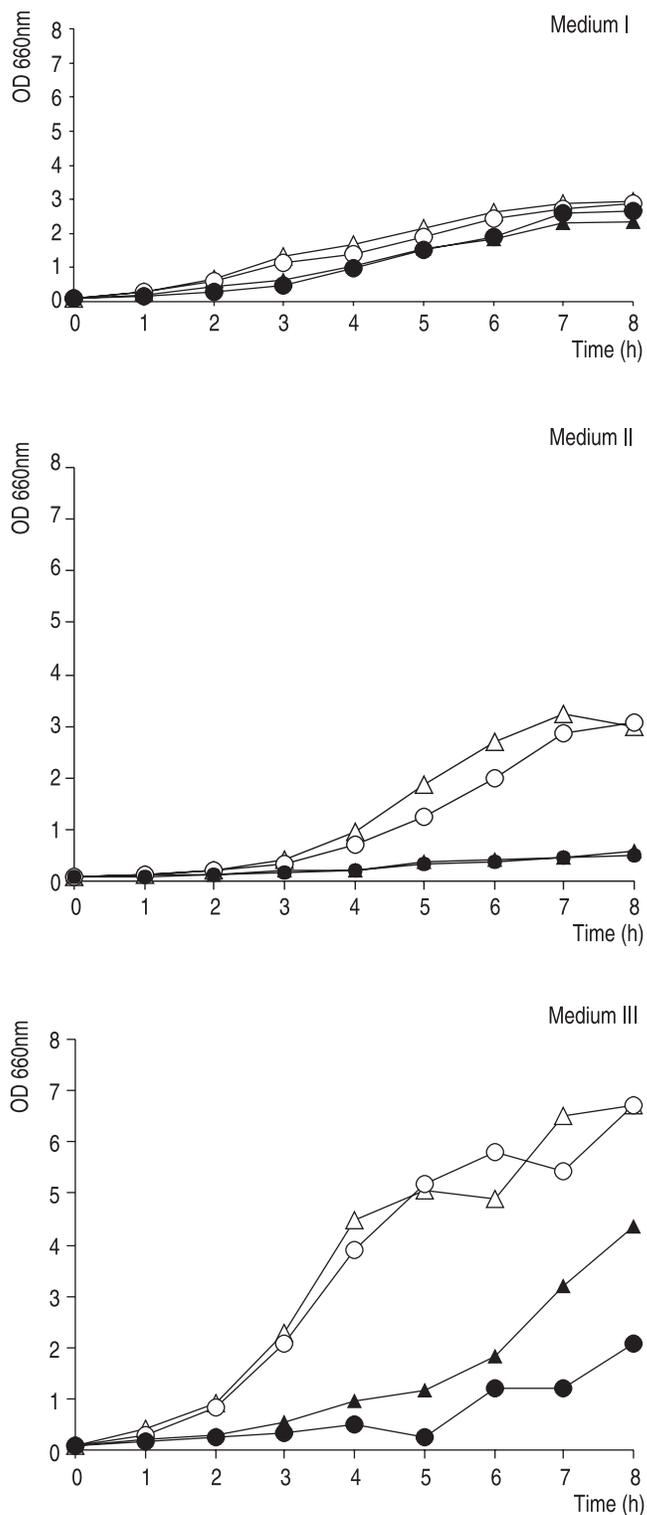


Figure 2. *E. coli* MKH13 + pOTPEX (circles) and MKH 13 + pOSEX (triangles) behavior during the culture in medium I, II and III. Non-induced cultures (opened symbols); induced cultures with 250 mM of NaCl (solid symbols).

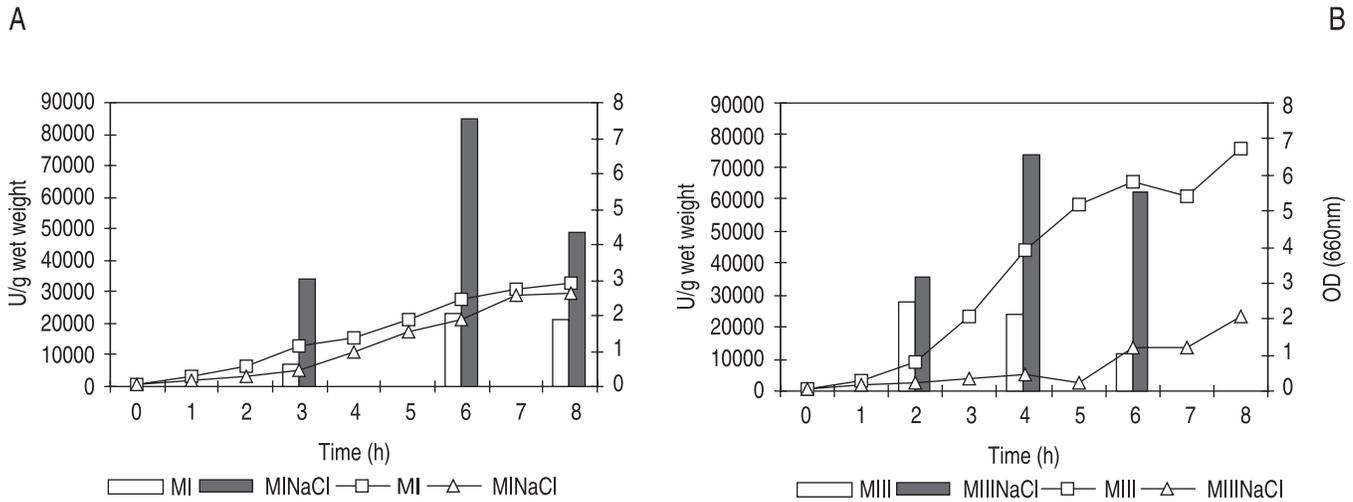


Figure 3. Growth curves and activity tested in induced and non-induced cultures of *E. coli* MKH13 (pOTPEX). medium I (A). medium III (B).

Activity was assayed in media I and III in different phases of growth and we obtained a maximum value in medium I at the end exponential phase (OD at A_{660nm} , ≈ 3.45). 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 (Fig. 3A). Similar results were obtained in medium III, but the greatest activity value was lower than in medium I (Fig. 3B). 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 (Fig. 3). *E. coli* DNA polymerase activity in MKH13 transformed with pOTPEX (negative control) was not detected in our polymerase activity assay.

Influence of NaCl concentration in cell growth and enzyme activity

The influence of different NaCl concentrations on enzyme activity in the same growth phase was analyzed in medium I. The growth decreased as osmolarity of the medium increased due to an increment in NaCl concentration (Fig. 4A). 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 disagreement with previous reports^{3,10} (Fig. 4B). 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 (Fig. 3).

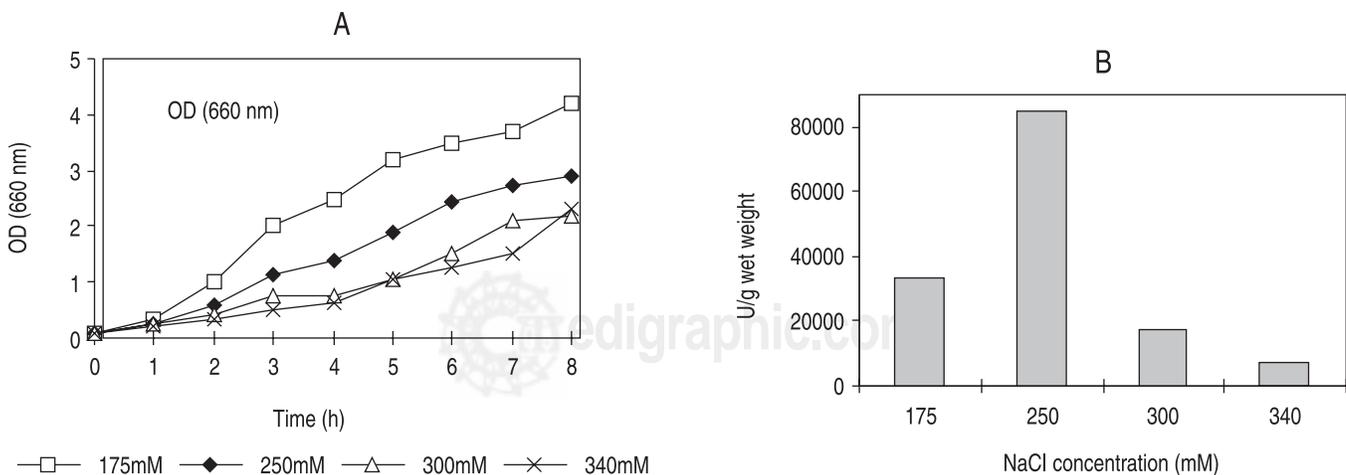


Figure 4. Effects of different NaCl concentrations on the cell growth (A) and Taq Pol I activity in the same phase of growth (B).

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