LONG-TERM CONTINUOUS ADAPTATION OF *Escherichia coli* TO CHEMICAL INDUCTION IN THE PRODUCTION A RECOMBINANT PROTEIN

María Fernanda Nava-Ocampo, David Fernando Zuluaga-Rave, Alejandra Dávila-Truby, María de los Ángeles Cancino-Rodezno, Norma Adriana Valdez-Cruz, Mauricio Alberto Trujillo-Roldán.

Departamento de Biología Molecular y Biotecnología, Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México. México D.F. CP 04360; maurotru@gmail.com

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Introduction. The production of recombinant proteins (RP) has become an area of interest in biotechnology since there is a great demand for this type of molecules. Usually, this proteins are produced in *E. coli* by the high specific growth rate and a plenty of good models of recombinant protein production. Chemical inductors that work at *lac* promoter level such as IPTG are highly used at laboratory level. Furthermore, the continuous cultures are used in the study of strains of adaptation to different selection pressures after several generations [4]. On the other hand, our model RP is an antigen of the pathogenic *Mycobacterium tuberculosis* known as Alanine-Proline rich Antigen (APA, also 45/47 kDa protein). This is a novel candidates as a component of a new vaccines or diagnostic kits against tuberculosis (TB) [1,2,3]. Since it is a human pathogen, culture *M. tuberculosis* to obtain its proteins is dangerous, as also the reduced specific growth rate (μ) and does not give high productivity.
The objective of this work is to analyze the production of the recombinant APA (rAPA) antigen and the plasmid segregation response to the adaptive behavior in continuous culture of recombinant *E. coli,* producer of APA antigen from *M. tuberculosis.*

Methods. Strain *E. coli* Rosetta (DE3) containing a pET-15b plasmid that express rAPA antigen. Cultures were carried out in a continuous mode using a 1.0 Liter bioreactor, in *Luria Bertani*, 0.68 mM ampicillin and 0.1 mM chloramphenicol. Feeding started at 3 hrs of batch culture and was fed with the same media above described plus 1.0 mM of IPTG. Dissolved oxygen was controlled at 30% by changing agitation speed, temperature was controlled at 30ºC and pH was not controlled (fig 1). Biomass growth was follow by optical density (fig. 1). The continuous culture was maintained at a dilution rate (D) of 0.03 h^{-1}. Production of rAPA was followed by SDS-PAGE and Western Blot. The number of copies of pET-15b during *E. coli* continuous culture was analyzed by RT-PCR for almost 210 generations, as also 2-D SDS-PAGE gels were done.

Results. Continuous culture with the addition of the chemical inductor (IPTG) was maintained with an average optical density of 6.24± 1.2 a.u. during 210 generations (4273 h). During 4200 h the protein was detected by SDS-PAGE and Western Blot. After this time, the production was not detected; this can be explained by the dilution of the plasmid (table 1).
The number of the copies of the plasmid per cell remains low during all culture, until the end (4273 h) that a complete loss of plasmid is observed (with a consequence loss of rAPA production).

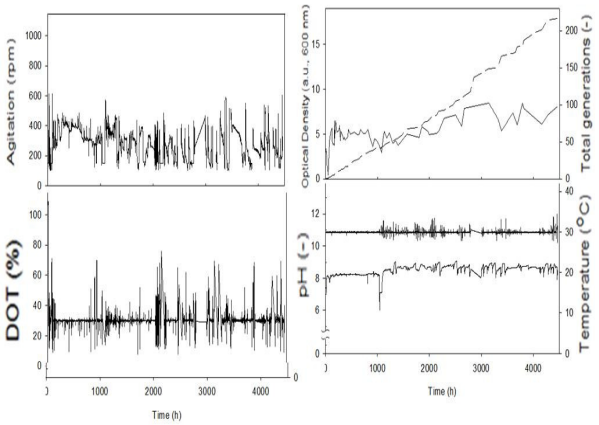

Fig.1 *E. coli* continuous culture conditions and biomass during 210 generations. Optical density (u.a.), Generations, DOT (%), Agitation (rpm), pH and temperature (ºC).

time (h)	pET-15B plasmid number/cell
zero	17.0
1110	1.16
2350	0.9
3428	3.4
4273	0.014

Table 1. Number of copies of the pET-15B plasmid during *E. coli* continuous culture by RT-PCR.

Conclusions. An adaptive test on APA strain producer under continuous induction with IPTG does not affect the recombinant production and is capable of keeping it constant even when the plasmid number per cell is considerably low.

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