Sociedad Argentina de Microbiología General

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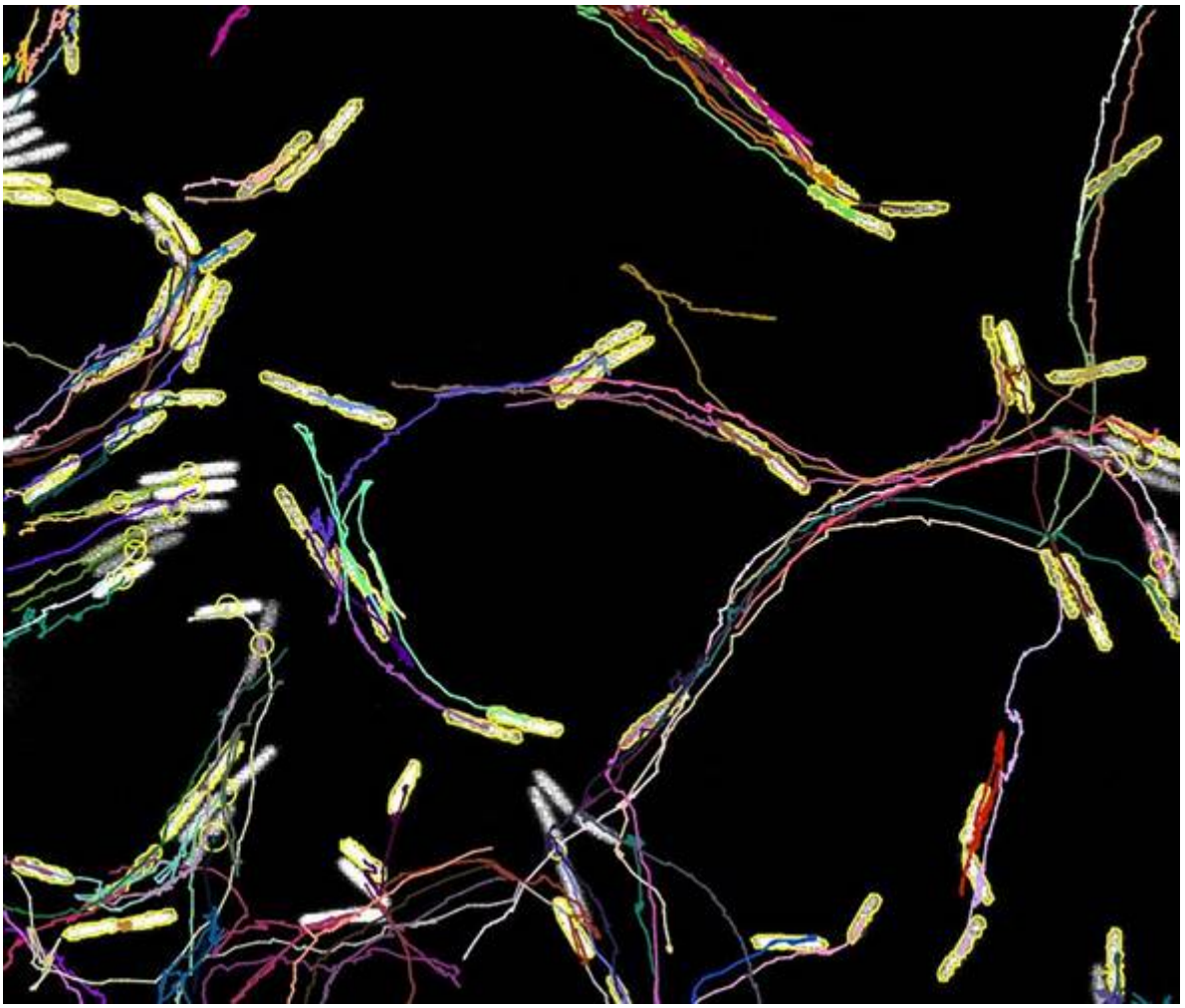

Foto: Se hace camino al andar. Celeste Dea. 1er puesto. Concurso fotográfico SAMIGE 20 años.

STUDY OF EXPRESION AND PURIFICATION OF THE RECOMBINANT HUMAN INSULIN GROWTH FACTOR

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The objective of this work was to study the expression and purification of the recombinant Growth Factor (FCr), analogue of human IGF, on an analytical scale. This growth factor is currently used as a supplement to defined, serum-free culture media in animal cell culture. In previous laboratory work, favorable results were obtained when evaluating the growth of animal cells in defined media developed by our group. These previous results suggest that the use of this FCr as an additional supplement is potentially favorable. Finally, the FCr production process was scaled to a preparative scale, in a bioreactor of 5 L. A relatively high amount of wet biomass (540 g) was obtained in fed-batch culture. *Escherichia coli* (*E. coli*) is the most used prokaryotic microorganism in the biotechnology industry for the production of recombinant proteins for diagnostic or therapeutic purposes. Among the advantages that this microorganism offers as a host compared to other systems, the following stand out: the level of knowledge about its physiology and genetics, easy genetic manipulation, high specific growth rate with respect to yeast and mammalian cells, high productivity of the recombinant in media. simple and low-cost cultivation methods, and which is a microorganism approved for the production of biopharmaceuticals. In the first instance, work was done on the generation of the master cell bank, the working cell bank and the productivity study of the recombinant *E. coli* clones obtained. Subsequently, the production of FCr continued on an analytical scale, in Erlenmeyer flasks, the recovery of the inclusion bodies produced and the optimization of the FCr purification process. Different washing conditions were studied (pH values 6.5, 7.5 and 8.5, presence/absence of 0.1% Triton and urea), and solubilization of the inclusion bodies (molar concentrations of urea 2 and 8 M and environmental conditions (room temperature and -20 °C). Molecular exclusion and ion exchange chromatographic techniques were carried out. The purification was monitored by absorbance at 280 nm, and by SDS-PAGE electrophoresis. Finally, the production process was scaled up, to a preparative scale, in a 5 L Sartorius bioreactor up to an OD = 63. An average specific growth rate of 0.7 h-1 for bath phase, 0.16 h-1 for fed batch and induction stage and wet biomass (540 g) were obtained for these fed-batch conditions. The master and working cell bank of the most productive recombinant strain was obtained (six

clones were selected). It was possible to express the FCr on an analytical scale and scale production as inclusion bodies. The recovery conditions were optimized, at analytical scale, for washing and solubilization of the inclusion bodies (washing buffer at pH 8.5 and solubilization buffer 8 M urea). In addition, the results obtained allowed the FCr production process to be scaled.

Palabras clave: Bioprocesses - Recombinant protein - Purification - Supplements