Sociedad Argentina de Microbiología General

XIX CONGRESO DE LA SOCIEDAD ARGENTINA DE MICROBIOLOGÍA GENERAL

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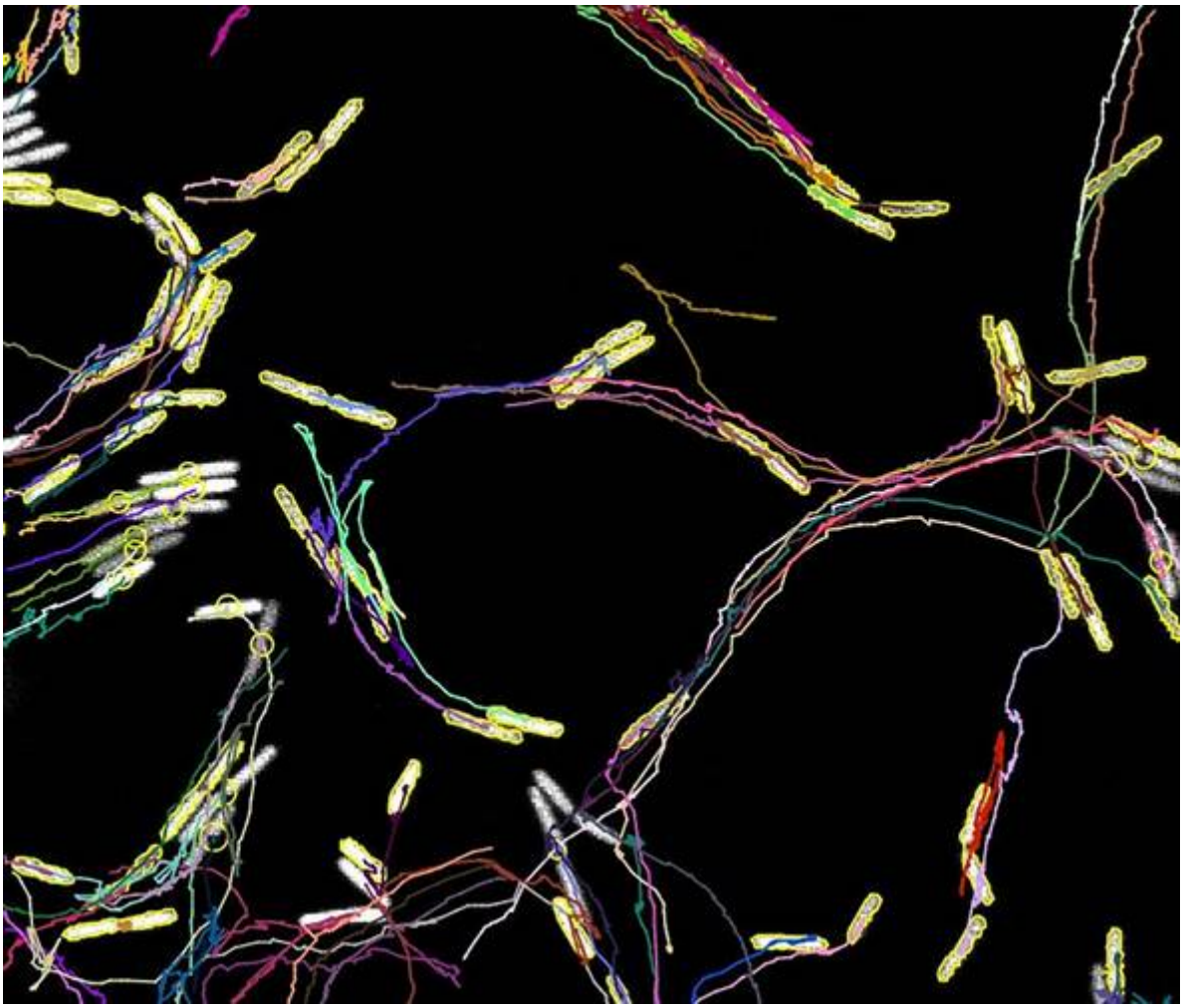

Foto: Se hace camino al andar. Celeste Dea. 1er puesto. Concurso fotográfico SAMIGE 20 años.

USE OF RECOMBINANT Staphylococcal PROTEIN A FOR IMMUNOGLOBULINS PURIFICATION

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Affinity chromatographic supports are nowadays one of the most frequently used and expensive consumable materials for protein purification at the laboratory and industrial scales. *Staphylococcus aureus* protein A (SpA) is a single polypeptide protein of 42 kDa with a high affinity for Fc region of immunoglobulins (IgG). SpA is the preferred ligand for binding antibodies and molecules tagged with an Fc region in several immunological and biotechnological applications, such as affinity chromatography and immunochemical techniques. Therefore, there is a need for high-level production of the protein. SpA can be obtained through the culture of wild-type *S. aureus*, but recombinant protein produced in *Escherichia coli* is convenient due to their biosafety and appropriate ligand design. This study focused on an engineered SpA-based ligand, AviPure [1] produced in *E. coli*. This recombinant protein was designed to improve specific interaction with IgG and to introduce two aminoacidic tags for purification and immobilization purposes. Though SpA has five domains with an affinity for the Fc region, the molecule is incapable of binding five IgG molecules due to steric hindrance. This problem was overcome by using AviPure, which has a lower molecular weight (14 kDa) and contains two SpA domains, a histidine tag at the N-terminal for Ni–IDA-based purification and a Cys tag at the C-terminal for an oriented immobilization in solid supports [2]. In this work, the immobilization of protein ligands onto chromatographic supports were investigated. The protocol was optimized based on the fluorescence properties of a green fluorescent protein (GFP) as a model [2], and the reaction was monitored by fluorescence microscopy. The immobilization procedure was applied to the immobilization of the AviPure ligand, and the resultant chromatographic matrix was tested for human immunoglobulin adsorption. AviPure was successfully immobilized on Eupergit C and Sepharose (ligand density 4 g/l, in both cases), and the matrices were analyzed for IgG purification. While Eupergit C-AviPure showed specific immobilization by the cysteine tag, the reaction with Sepharose was non-specific. However, the antibody adsorption capacity of the Sepharose matrix was much higher (7 mg of IgG per ml of resin) and it also proved to be very specific, having been tested with animal sera. [1] Kangwa et al (2015). AMB expr 5 (70). [2] Kikot et al (2014). J Mol Recognit 27(11). 659-68.

Palabras clave: Recombinant proteins - Escherichia coli - Protein A - Immunoglobulin purification