Production of chimeric monoclonal IgG1 in *Escherichia coli* against *Acinetobacter baumannii* BamA protein

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INTRODUCTION

- ***Acinetobacter baumannii*** is a gram-negative coccobacillus that causes nosocomial infections worldwide and belongs to the ESKAPE group. It is currently one of the highest priority pathogenic bacteria for which it is necessary to find new effective alternative therapies to the use of antimicrobials, and passive immunotherapy with monoclonal antibodies against its outer membrane protein BamA, which is extensively conserved among known strains, could be a solution.
- **Monoclonal antibodies** are conventionally produced using hybridomas, but this products could also be produced more efficiently using *Escherichia coli as* strains capable of producing this therapeutic agents have been developed. One example is the SHuffle2 strain, which presents deletions in the disulphide bridge reduction pathways (*trxB* and *gor*) and cytoplasmatic overexpression of disulphide bridge isomerases. Thus, given the production yield of *E. coli*, it could be a great tool to be widely used in the monoclonal antibody industry since this therapeutic agents are increasingly being used for multiple health and diagnostic applications.

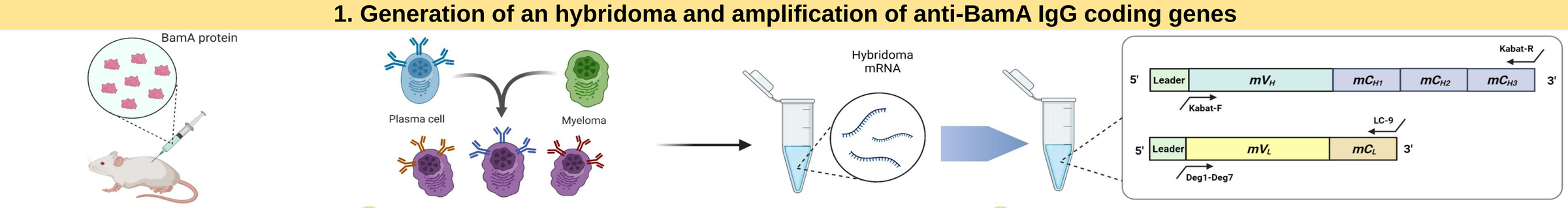
HYPOTHESIS AND OBJECTIVES

Production in Shuffle2 of aglycosilated IgG capable of binding specifically to the BamA protein of *Acinetobacter baumannii* and inducing its phagocytosis could show promising results in *in vitro* assays as a future passive immunotherapy treatment.

- **To establish a procedure that includes the use of *Escherichia coli* for the screening of the sequences of monoclonal, chimeric and aglycosylated IgG variable regions with affinity for the BamA protein of *A. baumannii.***
- **To test the recognition of this aglicosylated IgG by phagocytic cells of the human immune system.**

MATHERIALS AND METHODS

1. Generation of an hybridoma and amplification of anti-BamA IgG coding genes



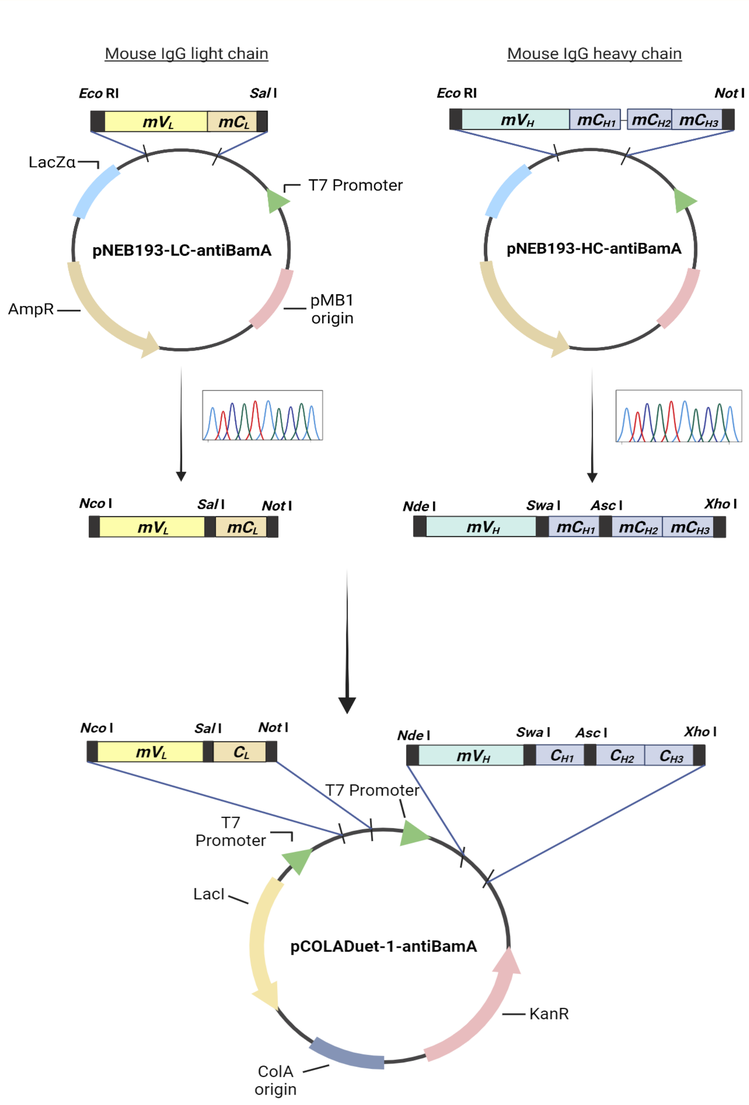
1.1 Immunization of mice with purified BamA protein to stimulate antibody production.

1.2 Isolation of antibody secreting plasma cells, fusion with myeloma cells and generation of hybridomas (selected in HAT medium).

1.3 Hybridoma mRNA extraction.

1.4 Conversion to cDNA and amplification of the heavy and light IgG chains without the leader sequence.

2. Cloning of the IgG coding genes in pCOLADuet-1 plasmids

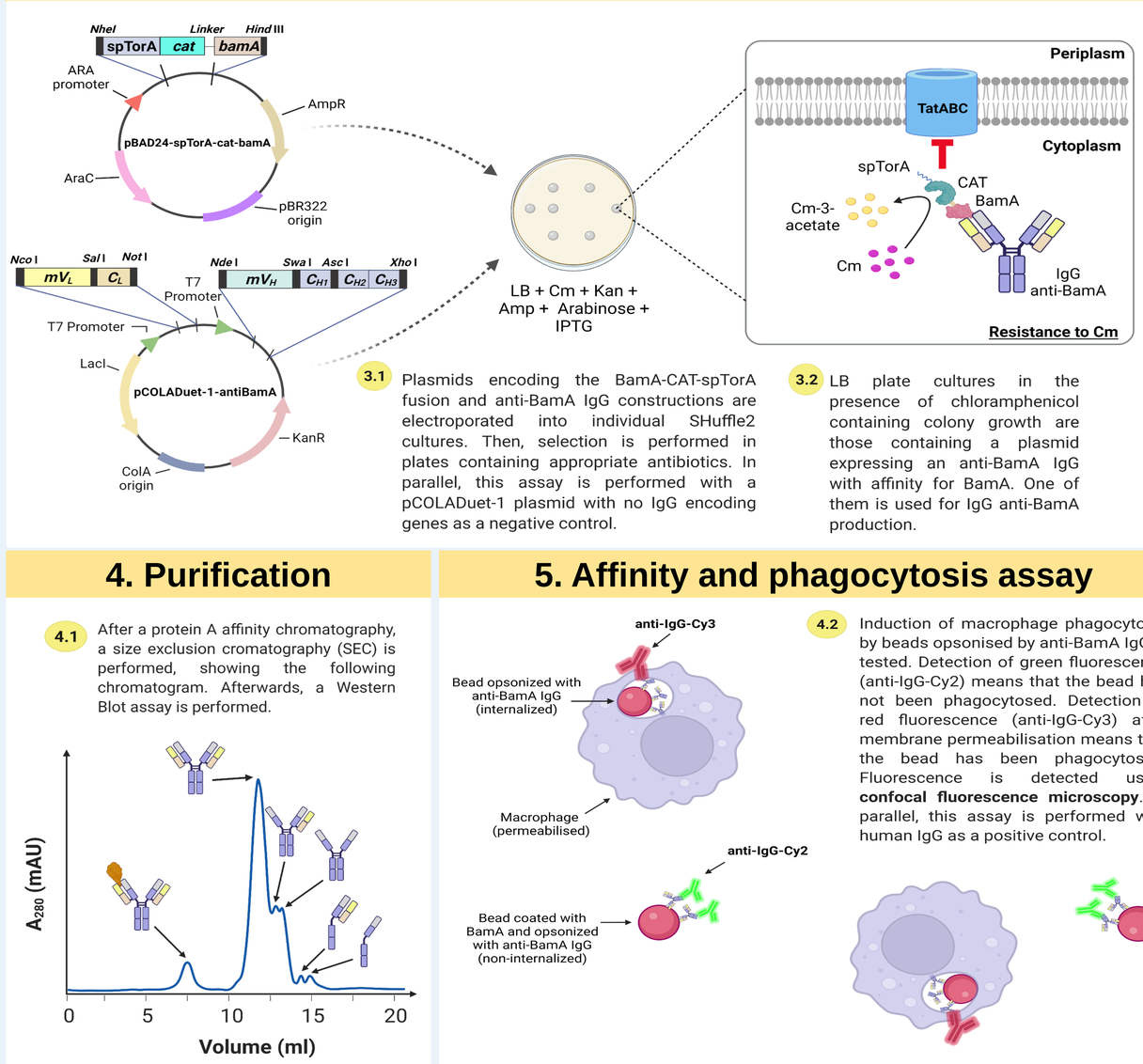


2.1 The amplified genes encoding the mouse IgG chains are cloned into pNEB193 plasmids for subsequent sequencing.

2.2 Additional restriction sites are added to the IgG genes by synthetic gene synthesis and overlap extension for cloning to the pCOLADuet-1 plasmid.

2.3 **pCOLADuet-1-antiBamA** plasmids are constructed and the genes encoding the constant regions of mouse IgG are replaced with the human constant regions (C_L, C_{H1-3}). Moreover, three mutations in C_{H1} and C_{H3} Fc encoding genes are introduced in order to favour antibody and phagocytes interactions: **E382V**, **M428I** and **V214 (GTG to GTT)**.

3. BamA-affinity IgG screening and production



3.1 Plasmids encoding the BamA-CAT-spTorA fusion and anti-BamA IgG constructions are electroporated into individual SHuffle2 cultures. Then, selection is performed in plates containing appropriate antibiotics. In parallel, this assay is performed with a pCOLADuet-1 plasmid with no IgG encoding genes as a negative control.

3.2 LB plate cultures in the presence of chloramphenicol containing colony growth are those containing a plasmid expressing an anti-BamA IgG with affinity for BamA. One of them is used for IgG anti-BamA production.

4. Purification

4.1 After a protein A affinity chromatography, a size exclusion cromatography (SEC) is performed, showing the following chromatogram. Afterwards, a Western Blot assay is performed.

5. Affinity and phagocytosis assay

4.2 Induction of macrophage phagocytosis by beads opsonised by anti-BamA IgG is tested. Detection of green fluorescence (anti-IgG-Cy2) means that the bead has not been phagocytosed. Detection of red fluorescence (anti-IgG-Cy3) after membrane permeabilisation means that the bead has been phagocytosed. Fluorescence is detected using **confocal fluorescence microscopy**. In parallel, this assay is performed with human IgG as a positive control.

EXPECTED RESULTS

- **To increase the availability of treatments that do not require antibiotic administration for *Acinetobacter baumannii* infections.**
- **Provide sequences of antibodies against the BamA protein to be studied for humanization.**
- **Motivate other scientists to start new projects in order to improve this strategy of monoclonal antibodies production.**
- **Application of the exposed procedure to the generation of monoclonal antibodies for other purposes.**

DISSEMINATION PLAN

- Publications in **biotechnology** and **applied microbiology** journals.
- Presentation of the poster at national and international conferences to show this alternative to the use of hybridomas.

REFERENCES

(1) Robinson MP, Ke N, Lobstein J, Peterson C, Szkodny A, Mansell TJ, et al. Efficient expression of full-length antibodies in the cytoplasm of engineered bacteria. Nat Commun. 2015 Nov;6(1):8072. (2) Lénon M, Ke N, Szady C, Sakhtah H, Ren G, Manta B, et al. Improved production of Humira antibody in the genetically engineered Escherichia coli SHuffle, by co-expression of human PDI-GPx7 fusions. Appl Microbiol Biotechnol. 2020 Nov;104(22):9693–706. (3) Lobstein J, Emrich CA, Jeans C, Faulkner M, Riggs P, Berkmen M. SHuffle, a novel Escherichia coli protein expression strain capable of correctly folding disulfide bonded proteins in its cytoplasm. Microb Cell Fact. 2012 May 8;11:56.(4) Choy CH, Botelho RJ. Quantifying Phagocytosis by Immunofluorescence and Microscopy. In: Botelho R, editor. Phagocytosis and Phagosomes: Methods and Protocols. **Images created with Biorender.com**