Design of a bacterial ghosts-based vaccine against *Staphylococcus aureus*

Research Project Proposal

Maria Peña Romero *Bachelor's degree in* ***Biotechnology*** *Universitat Autònoma de Barcelona*

1. Introduction

This poster is a Research Project Proposal for the design of a vaccine against *Staphylococcus aureus*.

S. aureus is a gram-positive bacterium, the most common cause of nosocomial infections. It is also resistant to most of the antibiotics. The design of an effective vaccine would reduce the amount of infected people, specially in hospitals.

2. Hypothesis

Escherichia coli's Bacterial Ghosts (BG) that express Fibronectin-binding protein A (FnBPA) in their surface will generate effective immune response against *S. aureus* infections.

Positive evidences were shown in a BG-based vaccine against *Helicobacter pylori.*

3. Objectives

To design a vaccine against *S. aureus* that:

a) Stimulates both cellular and humoral immune response.

b) Avoids secondary responses against the vaccination process.

What is an *E. coli* BG and which are their applications to this vaccine design?

BGs are generated by the outer and inner membranes of *E. coli* being lysate by the E- enzyme, which forms a tunnel that allows the bacterial cytoplasmic content go outside.

BGs work as carriers for a *S. aureus* virulence protein which is exposed in their outer membrane, FnBPA in this case. This structure will merge the bacterium natural infection.

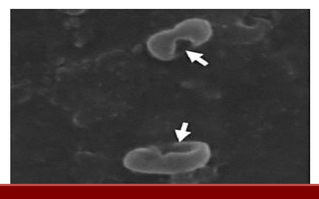


Fig 1: *E. coli* bacterial ghost characterized by Scanning Electron Microscope (SEM).

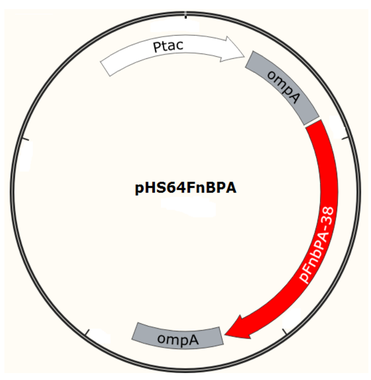
Why FnBPA?

- Fibronectin binding protein A is a *S. aureus* protein which allows the bacterium to adhere to hosts and enter inside them, acting as a bridge.
- FnBPA is involved in adherence to mammalian cells and in the infective process.
- In *Helicobacter pylori* a BG-based vaccine had positive results using a protein with a 70% of identity with FnBPA. This was determined by a BLAST analysis.

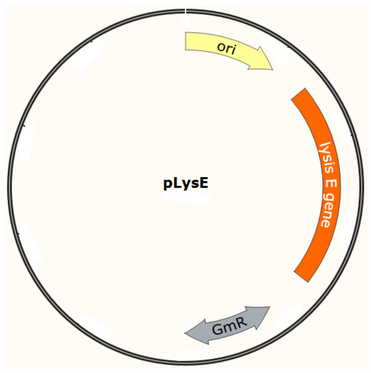
Materials and Methods

Plasmid constructions

pHS64FnbPA: 38 aminoacids of FnBPA were cloned into plasmid pHS64. FnBPA was flanked between two ompA (outer membrane protein A) sequences. This allows the expression of the FnBPA in the *E. coli* outer membrane. This construction is under a P_{TAC} promoter control.



pLysE: cassette with lysis E gene was cloned into plasmid pBBR1MCS5 with a replication origin and a gentamicin resistance. Its induction is under temperature regulation.



Vaccine production

1. *E. coli* is cultured in LB media at 28ºC and transformed with pHS64FnBPA and pLysE plasmids by electroporation. CFUs are counted.
2. pHS64FnbPA induction adding 0.5 mM IPTG: **ompA-FnBPA38-ompA insertion** in *E. coli* outer membrane.
3. pLysE induction raising temperature to 42ºC: **tunnel formation.**
4. Antibiotic addition to inactivate *E. coli* after transformation and after 1h, CFUs recount.
5. SDS-PAGE and Western Blot assay to determine if FnBPA is into the outer membrane

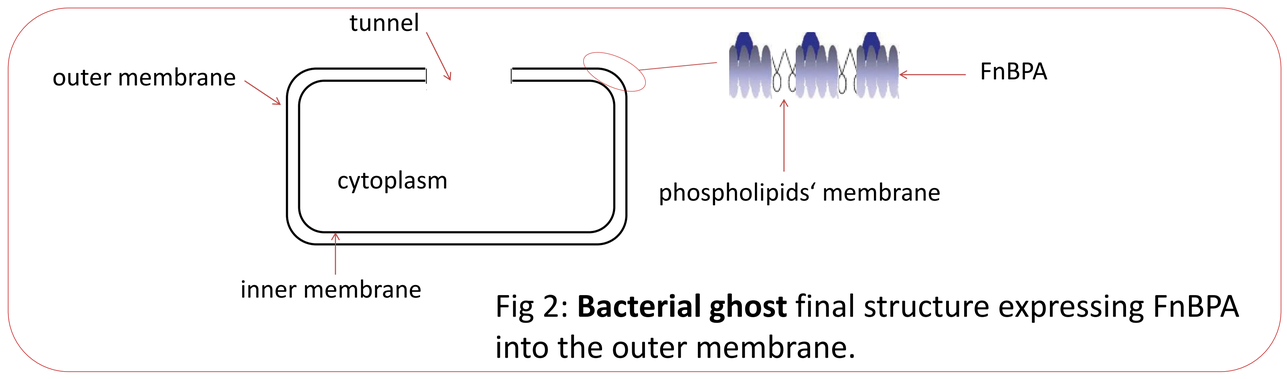
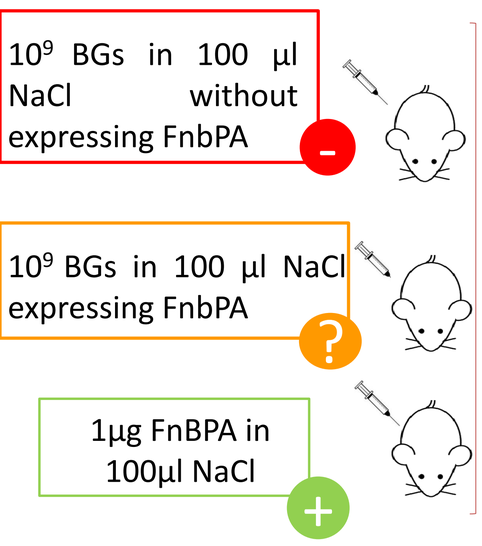


Fig 2: **Bacterial ghost** final structure expressing FnBPA into the outer membrane.

Immunogenic assays

28 weeks-old females BALB/c mice were immunized via subcutaneous injection (15 ♀ for each group)



2 days → Blood samples were analyzed by **ELISA test** to determine antibodies (Ab) presence.

14 days → **Boost dose** to 5 mice of each group. Second **ELISA test**

21 days → $5 \cdot 10^7$ CFUs injected to all mice to do a **surveillance assay**

8 weeks → Mice were **sacrificed.**

Survivor mice

Non-survivor mice

Spleens were lysed and stimulated with 30µg of sonicated *S. aureus* (+ negative control with concavalin A).

The presence of TCD4/8 was analyzed by **flow cytometry**.

Conclusions

After first immunization, FnBPA specific Abs are expected to be detected in both sample injection and positive control in the two ELISA test. If there is no presence, the boost dose (half of the initial dose) is supposed to solve this problem. FnBPA-Abs presence in negative control should to be zero.

TCD4 and TCD8 lymphocytes are also supposed to be detected in immunized mice using flow cytometry. The same response as in ELISA test has to be detected in negative control.

The CFUs concentration in the surveillance assay is supposed to kill the 80% of mice not immunized. Control and treated mice with the BGs carrying FnBPA are supposed to have higher surveillance rates than negative control. The best situation will be a 100% surveillance with the sample dose.

If this strategy works in mice it should be a good vaccine to be tested in human patients.

Relevant bibliography

1. CHEN J *et al.* Helicobacter pylori outer inflammatory protein DNA vaccine-loaded bacterial ghost enhances immune protective efficacy in C57BL/6 mice, Vaccine Journal nº 32 [6054-6060], 2014
2. JECHLINGER W *et al.* Comparative immunogenicity of the Hepatitis B virus core 149 antigen displayed on the inner and outer membrane of bacterial ghosts, Vaccine Journal nº23 [3609-3617], 2005.
3. BEATE MAYR U *et al.* Bacterial ghosts as antigen delivery vehicles, Advanced Drug Delivery Reviews, nº 57 [1381-1391], 2005.

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