Recombinant *Vibrio cholerae* ghost as a delivery vehicle for vaccinating against *Staphylococcus aureus*

Adrià Auladell Martín

Degree in microbiology, 2014. Universitat Autònoma de Barcelona

Universitat Autònoma de Barcelona

Initial hypothesis and objectives

The main objective of this project is to create a vaccine able to confer protection against heterologous strains of *Stahylococcus aureus*. It will induce a cellular immune response based on the polarization of CD4T helper cells to Th17 / Th1.
The initial hypothesis is that the Bacterial Ghost methodology used in the construction of the vaccine is successful, and that the selected antigens (IsdA, IsdB and SdrD) generate a specific protective immunity in the individuals who was administered.

Background

Staphylococcus aureus is a grampositive ubiquitous bacterium from the phylum Firmicutes. It is known to be present as a commensal in the skin and nasal microbiome in 25–30% of humans, and it is also a common pathogen, causing skin and soft tissue infections, such as cellulitis, impetigo, and folliculitis[1]. Although these infections are usually originated in the skin, invasive and life-threatening infections such as bacteremia, pneumonia, meningitis, endocarditis, toxic shock syndrome (TSS) and sepsis may ensue [2]. During the last decade, the treatment of these infections has been difficulted by the irruption of resistant strains, such as methicillin resistant strain (MRSA *S.aureus*) or vancomicin resistant strain (VRSA *S.aureus*) [3–5]. Due to this fact, in United States, *S.aureus* represents one of the major causes of death. Indeed, it is involved in over 18,000 deceases per year; about 290,000 hospitalizations and almost 12 million of medical visits and treatments [1].

Different studies established a link between Th17 cells and neutrophils. Interleukin 17A (IL-17A) is known to be important during the neutrophils' recruitment facilitating chemotaxis, eliminating the bacterium easily. Th1 polarization is also required for the neutrophils' activation at the infection side because, for instance, an intracellular stage is known to appear sometimes in *S.aureus* infections[2,9]. Finally, it has demonstrated by Stranger Jones *et al* that IsdA, IsdB and SdrD confer protection against *S.aureus*[14] .

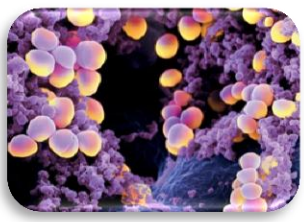
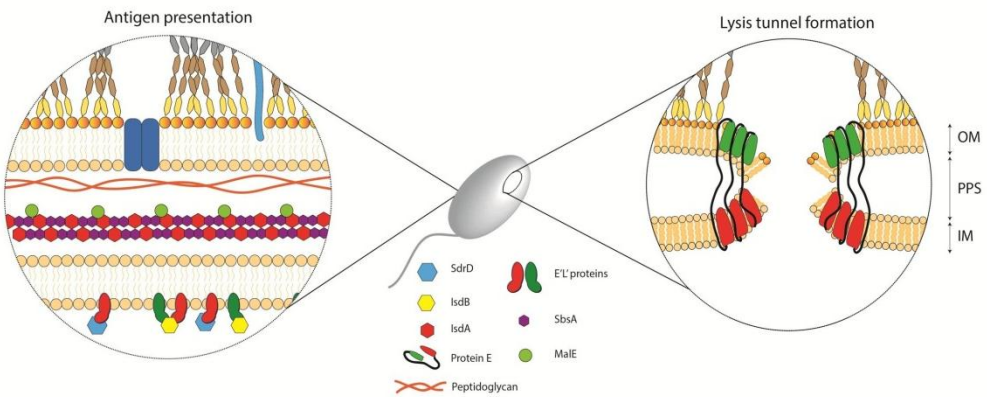


Figure 1: *S.aureus* developing a biofilm. Soucre: *Micronaut*



Bacterial ghost

Bacterial ghosts are empty cell envelopes produced from Gram-negative bacteria controlling E protein expression. This control is due to the cloned PhiX174 lysis E gene. E protein forms a tunnel structure spanning the whole cell wall complex, through which the cytoplasmic contents are expelled [10-12].
Recombinant DNA technology facilitates the development of multivalent protein or DNA vaccines. The expression of recombinant proteins can be localized in the **outer membrane** (OM), the **inner membrane** (IM) and the **periplasmic space** (PPS)[11].

Figure 2: Zoom into the bacterial ghost structure. We can see the IsdB and SdrD presentation by the E'L' proteins attached at the IM, and the IsdA presentation by the S-layer formation mediated by SbsA into the PPS (MalE delivers S-layer into the PPS). On the right side we can see the lysis tunnel formation by protein E.

Materials and methods

Obtaining of vaccine subunits

Chemical synthesis of the genes using *V.choleare* codon usage

Digestion of pMAL-A and IsdA with *Ifl III*

Digestion of pKSEL5-2, IsdB and SdrD with the respective restriction enzimes

Ligation (T4) and transformation into *E.coli* JM109

Selection of clones (LB agar + Ampicilllin + Cloramfenicol).

Confirmation by colony PCR, sequencing plasmids.

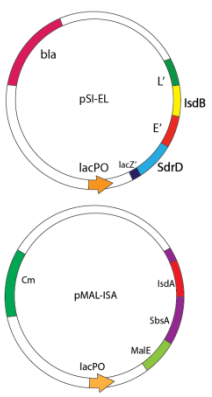


Figure 3: Scheme of the genic constructs expressing the recombinant proteins.

Bacterial ghost formation

Plasmids electroporation in *V.cholerae* H01
Selection of the clones (BHI +Amp + Cm)

Electroporation of pDKL01 Culture growth (BHI + IPTG + Antibiotics) until OD ^{600} of 0.3

Lysis induction with 3-methilbenzoate

Centrifugation and **recombinant V.cholerae ghosts (rVCG) selection**

Culture BG to confirm lysis efficiency (no growth expected)

Confirmation by Western blot analysis

Protection studies

5 to 8 week old BALB/c mice pathogen free will be used. They will be immunized 3 times one week in groups of 10:

Group 1: Intramuscular dose of 3 mg rVCG in 50 µl of PBS
Group 2: Im dose of 1 mg rVCG in 50 µl of PBS
Group 3: Im dose of 3 mg VCG in 50 µl of PBS
Group 4: Im dose of 1 mg VCG in 50 µl of PBS
Group 5: Im dose of 50 µl of PBS in 50 µl of PBS

ELISA to determine antibody titer, IL-17 and IFN-γ
Flow citometry analysis to determinate Th17/Th1 cells levels

Infection of mice with lethal *S.aureus* dose ($8.7X10^8$ CFU) and survival study

Statistical analysis (t student test)

Expected results

The vaccine formed by the rVCG expressing IsdA, IsdB and SdrD should provide an effective immune response against *S.aureus* and a memory response. The protective response must be mediated by Th17 and Th1 cells, increasing the production of IL-17 and IFN-γ in the vaccinated individuals compared to the control ones. This protection will be effective against *S.aureus* due to the use of three different antigens all of them present in most of the pathogenic strains. During the lethal challenge, a survival increase in immunized mice is expected.

Project dissemination

After the concession of the project and its realization, a patent application will be submitted. Once the patent is granted, the project will be disseminated to the scientific community through a paper publication in a high impact journal such as *Clinical and Vaccine Immunology* (CVI) or *Vaccine*.

Benefits

This project provides a direct solution to one of the major problems in public health of the developed world: *S.aureus* infections are increasing the morbidity and mortality every year having an special importance the MRSA strains infections. The creation of a feasible vaccine has failed so far because there was a lack of information about the immune response able to establish an effective clearance of the infection.

To sum up, this vaccine will be cheaper than other approaches because of the simple production, it does not depend on complex purification processes, and the stock produced can be liophilizated making cold storage unnecessary.

1. Zecconi A, Scali F. Staphylococcus aureus virulence factors in evasion from innate immune defenses in human and animal diseases. *Immunol Lett.* 2013;150(1-2):12–22 **2. Cho JS, Pietras EM, Garcia NC, et al.** IL-17 is essential for host defense against cutaneous Staphylococcus aureus infection in mice. 2010;120(5). **3. De Kraker MEA, Wolkewitz M, Davey PG, et al.** Clinical impact of antimicrobial resistance in European hospitals: excess mortality and length of hospital stay related to methicillin-resistant Staphylococcus aureus bloodstream infections. *Antimicrob Agents Chemother.* 2011;55(4):1598–605. **4. De Kraker ME a, Davey PG, Grundmann H.** Mortality and hospital stay associated with resistant Staphylococcus aureus and Escherichia coli bacteremia: estimating the burden of antibiotic resistance in Europe. *PLoS Med.* 2011;8(10):e1001104. **5. Boucher HW, Corey GR.** Epidemiology of methicillin-resistant Staphylococcus aureus. *Clin Infect Dis.* 2008;46 Suppl 5(Supplement_5):S344–9.. **6. Bagnoli F, Bertholet S, Grandi G.** Inferring reasons for the failure of Staphylococcus aureus vaccines in clinical trials. *Front Cell Infect Microbiol.* 2012;2(February):16. **7. Spellberg B, Daum R.** Development of a vaccine against Staphylococcus aureus. *Semin Immunopathol.* 2012;34(2):335–348. **8. Proctor R a.** Challenges for a universal Staphylococcus aureus vaccine. *Clin Infect Dis.* 2012;54(8):1179–86. . **9. Miller LS, Cho JS.** Immunity against Staphylococcus aureus cutaneous infections. *Nat Rev Immunol.* 2011;11(8):505–18. **10. Tabrizi CA, Walcher P, Mayr UB, et al.** Bacterial ghosts--biological particles as delivery systems for antigens, nucleic acids and drugs. *Curr Opin Biotechnol.* 2004;15(6):530–537. **11. Langemann T, Koller VJ, Muhammad A, Kudela P.** The Bacterial Ghost platform system: production and applications. *Bioeng Bugs.* 2010;1(5):326–36. **12. Eko FO, Witte a, Huter V, et al.** New strategies for combination vaccines based on the extended recombinant bacterial ghost system. *Vaccine.* 1999;17(13-14):1643–9. **13. Mazmanian SK, Skaar EP, Gaspar AH, et al.** Passage of heme-iron across the envelope of Staphylococcus aureus. *Science.* 2003;299(5608):906–9. **14. Stranger-Jones, Y. K., Bae, T., & Schneewind, O.** (2006). Vaccine assembly from surface proteins of Staphylococcus aureus. Proceedings of the National Academy of Sciences of the United States of America, 103(45), 16942–7.