Lactococcus lactis: a new typhoid vaccine

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Introduction

Typhoid fever, caused by *Salmonella enterica* serovar Typhi is a severe world health problem, and current vaccines are quite defective. In this work a completely new vaccine is proposed, based on the immunologic properties of Lactic Acid Bacteria. This vaccine is designed with the necessities of the population affected by the disease, including safety, economically viable production and biological containment system.

1. The Disease

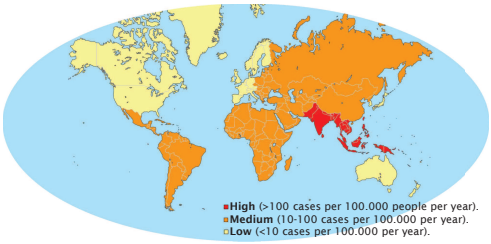


Figure 1. Incidence of typhoid fever. Data extracted from Crump et al. 2004 (1).

Typhoid fever is still a serious problem worldwide. Even though it's controlled in first world countries:, 216.000 deaths and 22 million cases are estimated per year (1). Cases of typhoid fever are more frequent in children and infants.

The host response is complex. To control de bacteria, it requires a T-cell response but also the production of antibodies (even though it's an intracellular pathogen).

Flagellin is a target of innate and adaptative immunity. This protein is recognized by innate immunity (throughTLR-5). It's also pointed to be recognized by T-cell and antibodies against flagellin are believed to be protective.
However, *Salmonella* has a flagellar phase variation between the two distinct flagellins FliC and FljB. Thus, *Salmonella* flagellins can be protective, but both flagellins must be present in the vaccination.

***Salmonella* Typhimurium is the murine model**. *Salmonella enteriva* serovar Typhi is the causative agent of typhoid fever but only infects humans. *S.* enterica serovar Typhimurium is the laboratory model in murine infection.

The two licensed vaccines are very defective. Meanwhile the attenuated strain Ty21a vacinne is not available for children under 6 years old, the capsular Vi polysaccharide vaccine elicits poor response in infants. Both vaccine have a poor rate of protection and require periodical re-vaccination.

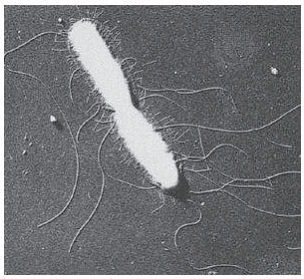


Figure 2. Salmonella typhiPhotography: J.P. Duguid and J.F. Wilkinson

2. *Lactococcus lactis*

Recombinant bacteria as vaccine vector have been studied since the early 1980'. Including pathogenic and non-pathogenic bacteria expressing heterologous antigens. Because of the hazard of reversion to a virulent state, non-pathogenic bacteria are a better candidate to treat typhoid, where children are the most affected subpopulation.

Lactic acid bacteria and *Lactococcus lactis* have been studied as vaccine vectors for long and there have been several protection trials in animal models (2). **Their benefits are**:

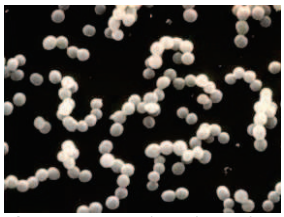


Figure 3. *Lactococcus lactis*. Photography: Bart Weimer, Utah State University

- ✓ **Safety**. They have been used for thousands of years in dairy food production and they are generally recognized as safe (GRAS).
- ✓ **Mucosal administration**. They can elicit an immune response through the natural route of infection of the pathogen. In addition, this avoids the use of needles and medically trained personal for its administration.
- ✓ **Inexpensive production**. As the bacteria are administered whole and alive, the downstream process in its production is much simpler than in other vaccines.

The immune response is hard to predict. The main drawback of Lactic acid bacteria vaccines is the dependence of many variables in the immune response elicited. This includes: strain, subcellular location of the antigen, the antigen itself, the animal model, and the route and regime of vaccination.

A biological containment system is needed using a genetically modified organism as vaccine vector. Thymidylate synthase gene (*thyA)* deletion is a bactericidal auxotrophy, so the generated strain requires a thymine or thymidine supplementation to survive. The gene substitution proposed is based on the work of Biswas et al. (3).

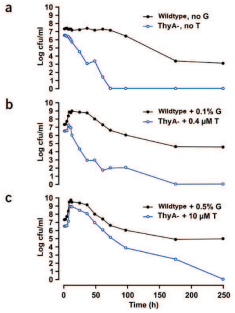


Figure 4. Growth of wildtype *L. lactis* strain and thyA- strain in different media. Data from Biswas et al. (4).

3. The vaccine

1. Plasmid Construction

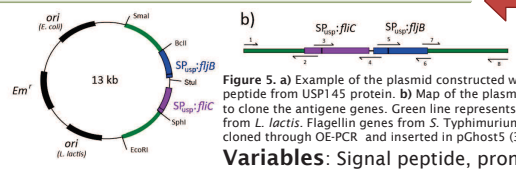
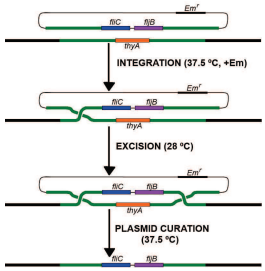


Figure 5. a) Example of the plasmid constructed with Signal peptide from USP145 protein. **b)** Map of the plasmids proposed to clone the antigene genes. Green line represents sequence from *L. lactis*. Flagellin genes from *S.* Typhimurium will be cloned through OE-PCR and inserted in pGhost5 (3).

Variables: Signal peptide, promotor

2. Bacteria recombination



The resultant plasmid will be transformed into *L. lactis*. The integration will be forced through temperature restriction of replication and selection. The excision will be improved through plasmid replication.

3. In vitro analysis

Confirmation
- Protein synthesis.
- Enough bacterial growth.
- Biological containment.

Unsatisfactory?

4. Animal immunization

As the immune response is hard to predict, immunization must be empirically found.

Variables: ammount (CFU/mice) and frequency of dose, route (oral/intranasally).

5. In vivo analysis

Quantification
- **Antibodies** (serum and mucosa)
- **Cytokines** (from ex vivo splenocytes and lymphocytes)

Unsatisfactory?

6. Prophylactic analysis

Lethal challenge: (with virulent *Salmonella Typhimurium*)
- Monitorization of mortality
- Organ examination.

The Benefits of this vaccine

Bibliography:

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2. Bahey-El-Din M, Gahan CGM, Griffin BT. Lactococcus lactis as a cell factory for delivery of therapeutic proteins. Current Gene Therapy. 2010;10(1):34-45.
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4. Steidler L, Neirynck S, Huyghebaert N, et al. Biological containment of genetically modified lactococcus lactis for intestinal delivery of human interleukin 10. Nat Biotechnol. 2003;21(7):785-789.