DEVELOPMENT OF ANTIGEN-DISPLAYING *BACILLUS* SPORES FOR ORAL VACCINATION AGAINST VIBRIOSIS AND MYCOBACTERIOSIS IN FISH

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NUTRIMU

INTRODUCTION

Incorporation of oral vaccines in fish feed

- Reduce fish stress
- Less vaccination costs
- Potential of using in fish larvae, juveniles, adults

Bacillus spores as antigen delivery vehicles

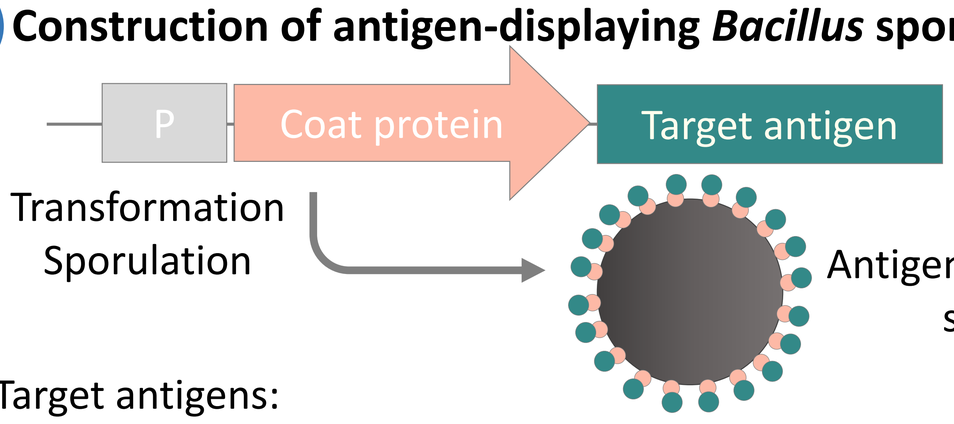
- Easy incorporation in feeds
- Heat stable
- Resistant to passage in the GI tract

OBJECTIVES

- Construction of ***Baciilus* spores** displaying target antigens from ***Vibrio spp.*** or ***Mycobacterium marinum***
- Development of zebrafish larvae **infection models** to test vaccine candidates

MATERIAL & METHODS

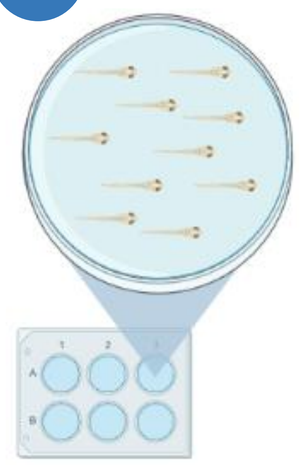
1 Construction of antigen-displaying *Bacillus* spores



Target antigens:
Vibrio spp. VAA and TolC
M. marinum Esx-1 and Ag85A

Probiotic *Bacillus* strains:
FI314, FI330 and FI442

2 Zebrafish larvae infection models



9dpf larvae challenged by immersion with different concentrations of:
Vibrio harveyi
Vibrio parahaemolyticus
Vibrio vulnificus

RESULTS

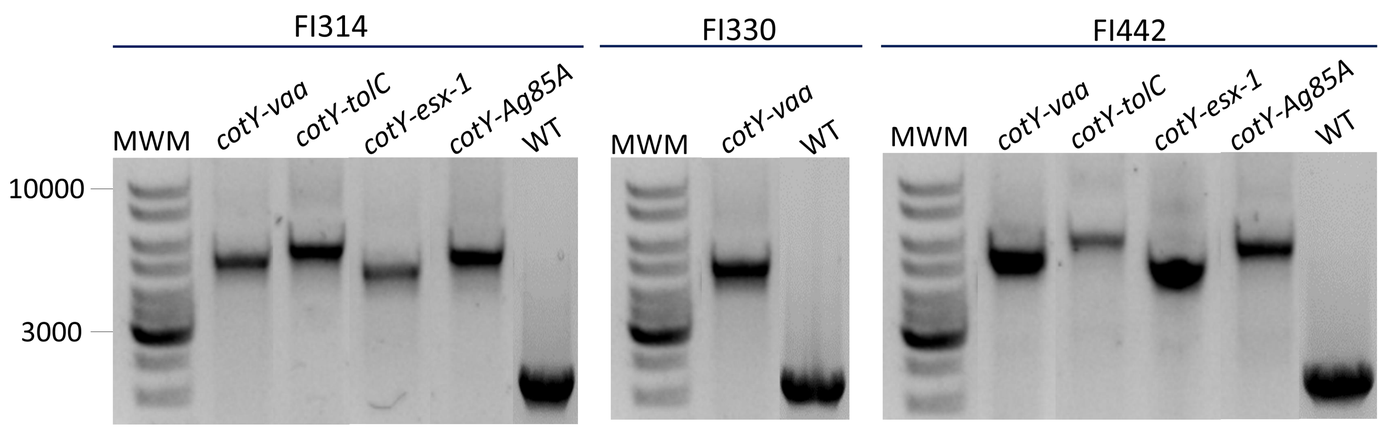


Fig. 1 Amplification of the *amyE* region, where a double-crossover event occurred to integrate the target fusions upon bacterial transformation. Bands of 4308 bp (*cotY-vaa*), 5037 bp (*cotY-tolC*), 4083 bp (*cotY-esx-1*), and 4680 bp (*cotY-Ag85A*) were obtained when using DNA from recombinant strains, confirming the successful integration of each fusion at the target *amyE* locus. DNA from the parental WT strain (FI314, FI330 or FI442) was used as control.

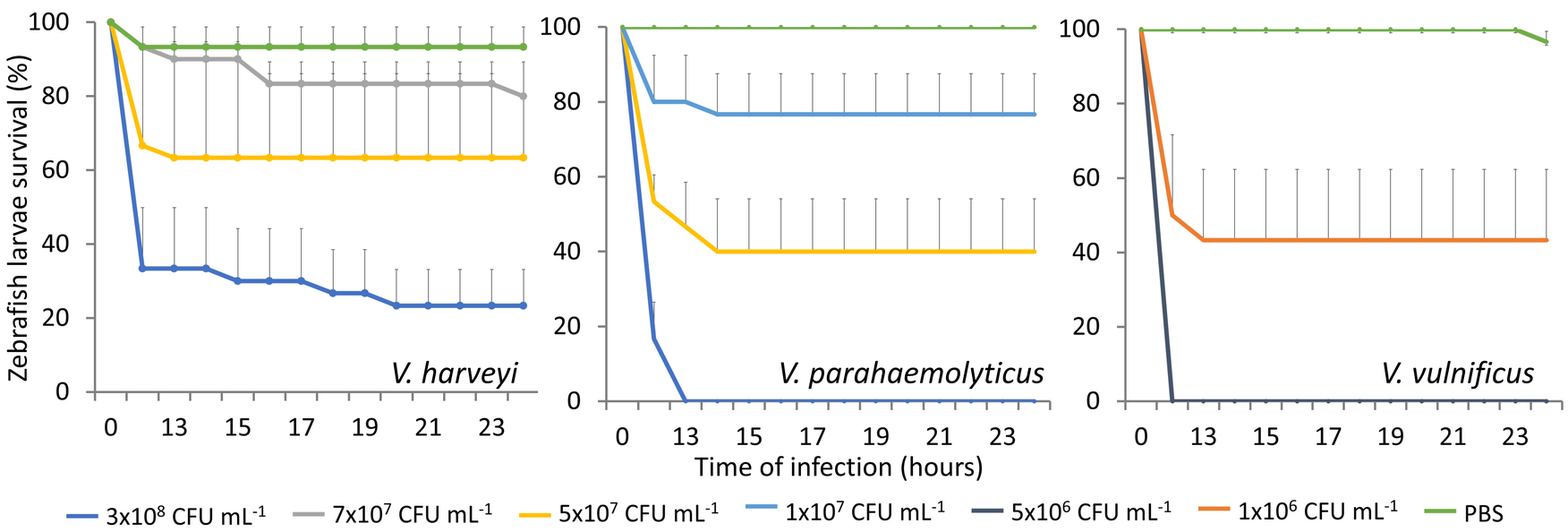


Fig. 2 Establishment of zebrafish larvae infection models. 9dpf zebrafish larvae were challenged with different concentrations ($1x10^6$ CFU mL^{-1} to $3x10^8$ CFU mL^{-1}) of *V. harveyi*, *V. parahaemolyticus* or *V. vulnificus* by immersion, and mortality was followed for 24h. Larvae exposed to PBS (1x) were used as negative control. Data shown are the mean of three independent experiments and the standard error of the mean is represented for each concentration. Survival of 30% to 50% was considered a criteria for choosing the pathogen concentration to be used in further studies to assess the protection potential of the antigen-displaying spores developed.

CONCLUSIONS

- ✓ *Bacillus* **FI314** and **FI442** were successfully transformed with fusions encoding **four target antigens - VAA** and **TolC** from *Vibrio spp.* and **Esx-1** and **Ag85A** from *Mycobacterium marinum*.
- ✓ Zebrafish infection models allowed the selection of the appropriate bacterial concentrations for further challenge studies.

FUTURE WORK

- ✓ Assess the protective effect of the recombinant spores using the established infection models.
- ✓ Establish an infection model for *Mycobacterium marinum*.

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