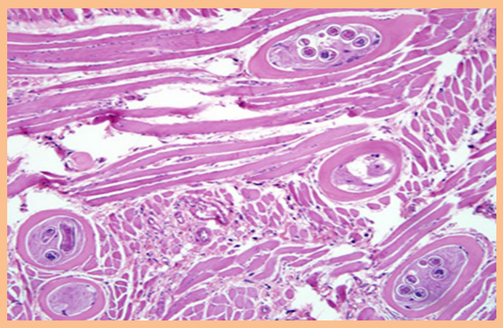
Development of a Novel Vaccine Delivery Platform Using Probiotic Yeasts



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Introduction

- *Trichinosis*, a re-emerging parasitic zoonotic disease, is a serious cause for concern in several countries.
- The most common causative agent is, *Trichinella spiralis*, a nematode which can complete all stages of a life cycle within a single host.
- *Trichinosis* is most often caused by consumption of muscle encysted larvae in undercooked pork and exerts significant burden, both clinically and financially, on affected populations. Thus the need for affordable and safe preventative measures is imperative.
- Many current vaccines do not induce long term protective immunity and , those which are successful, are costly in manufacturing and distribution.
- Prior mucosal vaccine development studies have found that stimulating the gut immune system can induce long term protective immunity locally and systemically (Fujkuyama *et al*., 2012).
- Many studies have focused on bioengineering probiotic bacteria as a delivery agent for vaccines. However, prokaryotes lack the complex machinery required for efficient and correct folding of proteins in eukaryotes.
- *Saccharomyces boulardii* is a eukaryote and well characterized probiotic yeast. It is generally regarded as safe, known to modulate host immunity within the gastrointestinal tract (GIT) (Martins *et al*., 2009) and is almost genetically identical to baker's yeast *Saccharomyces cerevisiae*.
- We are developing genetically modified (GMO) strains of *S.boulardii* as eukaryotic expression vectors for the delivery of mucosal vaccines to the GIT.

Aim

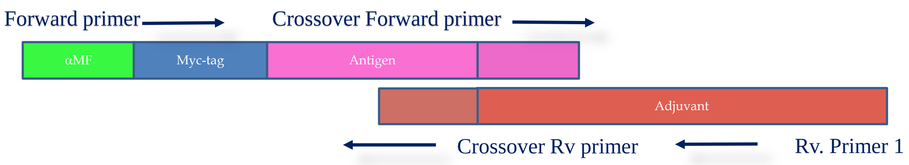
Construct a GMO *S.boulardii* strain expressing protective antigens targeting *T. spiralis* creating mucosal vaccine.

- Synthesize a chimeric DNA molecule containing i) α-mating factor (αMF); a secretion factor ii) epitope tag – for detection of the recombinant protein iii) *Trichinella* antigen 1 or 2 ,and iiii) protein adjuvant – to increase the immune response thus inducing memory. A cross-over PCR technique was employed.
- Clone into pGEM-T easy vector (Promega).
- Clone into the yeast expression vector pAC-TEF which will drive the expression of the chimeric protein with the strong constitutive TEF promoter.

Methods

•Individual products were first obtained using a typical PCR reaction at 50 degrees with appropriate forward and reverse primers used.
• Products linked using a crossover PCR reaction whereby templates were allowed to anneal in the absence of primers. The length of this varied from 7-10 cycles dependent on the size of the templates being joined.
•Primers with homologous regions to both templates were added in the second PCR programme to generate a final product consisting of one long open reading frame (ORF) and restriction sites (see figure 1A).

A.



B.

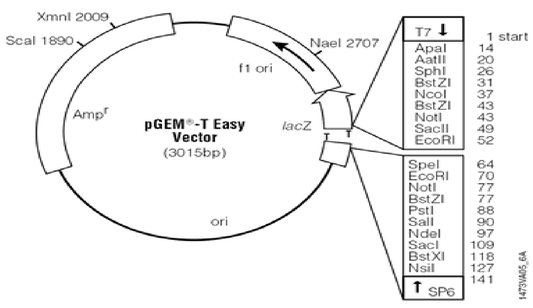


Figure 1. (A) Construction of chimeric protein generated by cross-over PCR- sequences were joined together sequentially using a molar ratio of 1:1 **(B)** Vector map of pGEM-T showing restriction sites.

•Recombinant DNA constructs were visualized by agarose gel electrophoresis.
•Products were gel extracted, a-tailed and ligated into pGEM -T Easy (see figure 1B), transformed into XL1 blue cells and plated on LB agar with ampicillin, x-gal and IPTG for blue/white selection. Colony screening and next generation sequencing confirmed the presence of the desired insert.

Results

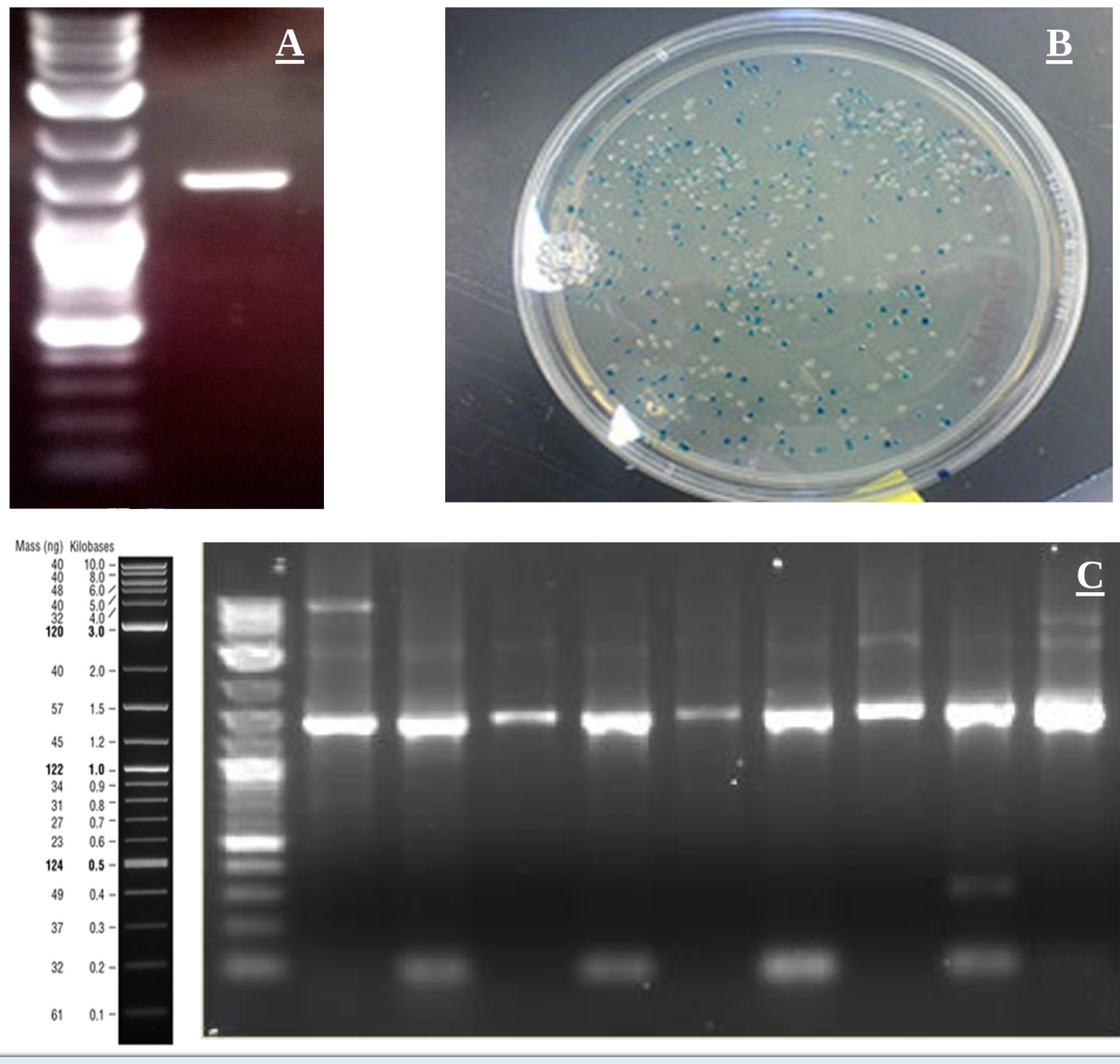


Figure 2. **A**, PCR product – chimeric DNA sequence at 1350bp, consistent with expected size. Run on a 1.5% Agarose gel. **B,** Blue/white colonies plated on LB agar + ampicillin, X-gal and IPTG. Transformed colonies containing insert are seen as white. **C**, Colonies positive for the final crossover insert in pGEM-T are seen above as bands at 1350 base pairs. Products were obtained using a Qiagen Plasmid mini prep and sent for sequencing using M13 forward and reverse primers.

Discussion

- DNA sequences from both the α-mating factor signal sequence/epitope tag and antigen were successfully joined using cross-over PCR (Figure 2) after various adjustments to a) number of PCR cycles, b) annealing temperature and c) extension time.
- Optimum parameters for the initial crossover cycle were 10 cycles of annealing and extension with an annealing temperature of 52 degrees and extension time of 2 minutes.
- The protocol for joining the DNA sequences was principally empirical as there is a distinct lack of previous literature on the subject. Published protocols focus on introducing mutations to the sequences by overlap extension PCR (Cha-Aim *et al*., 2012), however, this was used as a basis for the method.
- Products generated by PCR were ligated into pGEM-T effectively (Figure 3) whereby colonies were screened by PCR using sequence specific primers (Figure 4.).
- The second antigen, although visible on a 1.5% gel at the correct size (not shown), could not be joined to
- αmf/epitope-tag without a number of non-specific bands also being co-amplified and this is an issue that needs to be addressed in future work.

Future Work

- Sub-clone chimeric products into pAC8TEF, a yeast vector, for transformation into *S.boulardii*
- Detect expression of chimeric proteins using western blotting.
- Optimise expression of recombinant proteins using constitutive promoters.
- Determine type of immune response to vaccine in GIT using immunoassays such as ELISA.

References

Cha-Aim, K., Hoshida, H., Fukunaga, T and Akada, R. (2012) 'Fusion PCR via Novel Overlap Sequences', *Methods in Molecular Biology* 852 pp. 97-110 [Online] DOI: 10.1007/978-1-61779-564-0_8 (Accessed 2nd September 2014).
Fujuyama, Y., Tokuhara, D., Kataoka, K., Gilbert, R, S., Mc Ghee, J, R., Yuki, Y., Kiyono, H and Fujihasji, K. (2012) 'Novel Vaccine Development Strategies for Inducing Mucosal Immunity', *Expert Review of Vaccines*, 11 (3) pp. 367-79 [Online] DOI: 10.1586/erv.11.196 (Accessed 30th August 2014)
Martins, F, S., Silva, A, A., Vieira, A, T., Barbosa, F, H., Arantes, R, M., Teixeira, M, M and Nicoli, J, R. 2009. 'Comparative study of Bifidobacterium animalis, Escherichia coli, Lactobacillus casei and Saccharomyces boulardii probiotic properties', *Archives of Microbiology*, 191 (8) pp. 623-30 [Online] DOI: 10.1007/s00203-009-0491-x (Accessed 30th August 2014)