COMBATING SHIGA TOXIN

A Synthetic Biology Approach

Team Indian Institute of Technology Madras_iGEM 2013

INTRODUCTION

Motivation

Shiga toxin is produced by *Shigella dysenteriae* and a few strains of *E. coli* collectively called STEC (Shiga toxin-producing *Escherichia coli*), mainly comprising of strains like O157:H7, O111 and O26. Shiga toxin has a molecular weight of about 70 kDa. The toxin is produced after the bacteria colonize the cattle GI tract and form a biofilm. Shiga toxicity results in complications like bloody diarrhoea, abdominal cramping and fever in initial stages, but may lead to deadly diseases like hemorrhagic colitis, Hemolytic Uremic Syndrome(HUS) and kidney failure.

STEC and hence shiga toxin is transmitted primarily through unprocessed meat, raw milk and can be transmitted through contaminated food or water through fecal-oral route. Having claimed over 1 million lives worldwide, currently there is no comprehensive cure for the ailment and usasge of antibiotics only increases severity. Supportive care requires maintenance of fluid and electrolyte levels, and to monitor and support the kidney function.

Approach

In our project, we propose a novel two-fold Synthetic Biology approach to combat the lethal effects of the toxin:

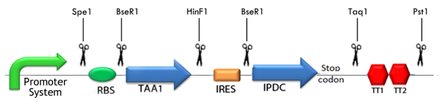
1. Neutralization of the toxin by a genetically engineered 9 amino acid Gb3 mimic peptide.
2. Inhibition of biofilm formation by production of indole-3-acetaldehyde in *E. coli* which in turn leads to inhibition of toxin production itself.

DESIGN

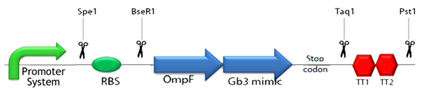
Constructs

Promoter System: AHL sensitive promoter (BBa_F2620) was used as the promoter system.

I3A production system: AHL sensitive pLuxR promoter upstream of polycistronic construct of *Tyrptophan Transaminase (TAA1)* and *Indole Pyruvate Decarboxylase (IPDC)*.



Gb3 mimic system: AHL sensitive pLuxR promoter upstream of *OmpF* sequence fused with *Gb3 mimic* sequence for extracellular expression.

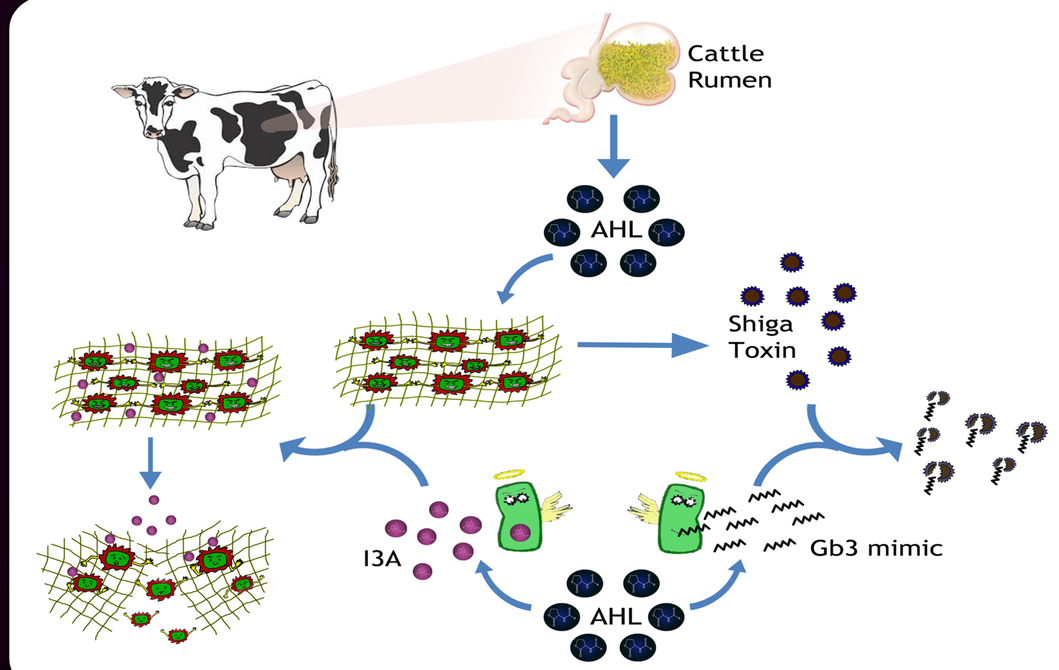


Modular Plug-and-Play System

We have designed the constructs such that each modular unit is followed by a Restriction Enzyme site. This design provides a unique feature of splitting the construct into different functional components. Thus, it offers great flexibility to tweak the system depending on the user's requirements.

ABSTRACT

Shiga toxin, a worldwide menace, has killed over 1 million people to date and continues to afflict almost 100,000 people each year. Currently, there is no treatment for Shiga toxicosis and it leads to complications in the human system like Hemolytic Uremic Syndrome (HUS) and renal failure. Toxin production depends mainly on biofilm formation of strains of *E. coli* like O157:H7 which in turn depends on the N-acyl homoserine lactone (AHL) concentration in the rumen. Hence, we propose a two-fold approach to combat the Shiga toxicosis. In this study, we have engineered *E. coli* to produce both an anti-toxin peptide (WHWTWLSEY) that is a (globotriaosylceramide) Gb3 mimic and a potent O157:H7 specific biofilm inhibitor, indole-3-acetaldehyde (I3A) in correlation with the concentration of AHL in cattle rumen. Our findings shed light on the importance and potential use of synthetic biology to combat such global health hazards.



OUTREACH

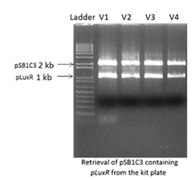
Project Code Red: We initiated a mission - *Project Code Red* - which aims at educating the cattle farmers, local and small slaughterhouses, butchers and consumers alike, about the risks associated with consumption of meat which may not be well processed, treated, packaged and cooked.

Distribution drives: We went around the city looking out for local butcher shops and slaughterhouses to distribute our comprehensive guidebook on safety issues involved in meat handling. We believe that this initiative would not only inform local meat vendors about the health hazards associated with contaminated meat but also provide them with suggestions to incorporate safe practices in their daily work.

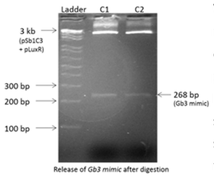
Outreach: We presented our project to students at highschool and undergraduate levels as an example of the tremendous potential that synthetic biology possesses in the field of Biotechnology, particularly in Health and Medicine.

RESULTS

Cloning



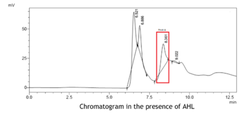
Retrieval of pSB1C3 containing *pLuxR* from the kit plate



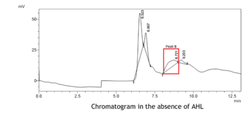
Release of *Gb3* mimic after digestion

We successfully engineered Gb3 mimic sequence (BBa_K1194000) downstream of AHL induced promoter system (BBa_F2620). Results were further confirmed by sequencing. Engineering of the I3A system was accomplished and further confirmation is being done by sequencing.

Reverse HPLC



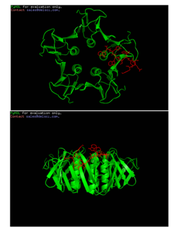
Chromatogram in the presence of AHL



Chromatogram in the absence of AHL

The chromatograms above show a significant difference in the height of the peaks (A & B). Peak A corresponds to the AHL induced sample while Peak B to the uninduced sample. We can state, with some confidence (n=8) that the peak A corresponds to the Gb3 mimic peptide.

Modeling



The web server PEP-FOLD was used to model the peptide (WHWTWLSEY) and HADDOCK was used to perform the docking studies.

Amongst the results predicted by HADDOCK, the cluster with the predicted HADDOCK score of -100.8+/-6.2 seemed to be the most suited interacting partner to Shiga toxin. This can be attributed to the optimum energy values assigned by HADDOCK as well as the high similarities of the binding modes of the peptide to the toxin as compared to that of the same of Gb3-Shiga toxin complex. This was analysed by the RMSD values as indicated by Pymol following structural alignment. The RMSD value obtained was 0.359.

FUTURE DIRECTIONS

Probiotic Encapsulation: To administer the engineered bacteria to cattle, via polymer encapsulation.

Direct drug delivery of anti-toxin: Based on further studies of bioavailability, stability and side effects, the polypeptide Gb3-mimic could be administered directly as a drug.

Peptide LIbrary: To construct a library of peptide mutants and analyse their interactions with Shiga toxin and Shiga-like toxins, and potentially extend our solution to similar toxicoses.

REFERENCES

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