**Lysis of shiga toxin-producing Escherichia coli by delivery of Crispr/Cas9 cascade coding cosmid targeting Stx 1 and Stx 2 genes via lambda**

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Introduction

Consumption of food or water contaminated with specific strains of *Escherichia coli* can cause dire illness and lead to development of more serious diseases such as hemolytic-uremic syndrome (HUS). *E. coli* strains that can express the shiga toxin gene (*Stx* *1* or *Stx* 2) are responsible for causing this foodborne illness, serotype O157:H7 being one of the most common. Of those who become infected with shiga toxin-producing E. coli (STEC), 5%-10% are expected to develop HUS, especially children and elderly persons [1].

*E. coli* become capable of producing shiga toxins when a bacteriophage that contains the gene for Stx integrates its own DNA into the genome of the *E. coli*, a process known as lysogenesis [2]. Stx genes are part of the late gene regions of the prophage – phage genome integrated into the host’s – which are genes expressed only after the induction of the lytic cycle [3]. After induction of the lytic cycle, early phage genes are transcribed which code for proteins that allow RNA polymerase to bypass transcription terminators of later genes, allowing them to be expressed. Once the shiga toxin is secreted out of the *E. coli*, it may invade surrounding tissues by passing through or in-between cells, or translocation through intact epithelial cells [4]. The toxin can travel to the kidneys, where they are taken up by cells with globotriaosylceramide (Gb3) receptors, and once in the cell, the toxin inhibits the host’s protein synthesis [5]. This proapoptotic stimulus is thought to cause HUS.

There are no current methods of treatment when sick with STEC, so it’s important for precautions to be taken at the source. There are several methods of prevention that include exposure reduction, exclusion reduction and direct anti-pathogen strategies. Exposure reduction strategies include providing cattle with clean water, feed, and a spacious environment to limit interaction between cattle. An example of exclusion reduction is use of chlorine treatment in water to lower transmission of pathogens. Water and feed additives are utilized for prevention as well. Adding antibiotics and probiotics to feed has been shown to decrease E. coli O157:H7 shedding. [6]

Taking antibiotics while sick with STEC is advised against. Antibiotic attacks on the cell and its DNA lead to an SOS response when transcription is increased. This SOS response can lead to an excess of shiga toxin production, which can increase the permit damage of having this pathogen. For many years, it was debated whether or not antibiotics caused this response due to mixed results from many experiments. It was found that various antibiotics have differing effects, depending on the strain of *E. coli* causing the infection [7]. This, coupled with depletion of the host’s microbiome, is why it is best for antibiotics to be avoided.

Here, a method of possible treatment of shiga toxin-producing *E. coli*-related illness is designed, one that could attack STEC cells without triggering stx gene transcription. Utilization of a Crispr/Cas9 system will allow for recognition and cutting of a specific DNA sequence, in this case, the shiga toxin gene. Delivery of a cosmid coding for a Crispr cascade via a lambda phage delivery system allows for selective infection of bacteria of the host’s microbiome, only targeting *E. coli*. While this would allow infection of harmless e coli cells as well, since they do not contain the Stx gene, their DNA will not be cut, avoiding cell lysis when the Crispr cascade has been translated.

Experiment

In order to control transcription of the Crispr/Cas9 genes in our cosmid during construction of these phage delivery systems, there needs to be a repressor present. The plasmid pACYC184 can be edited to replace the region between HindIII and AvaI with a Tet repressor gene. The main cosmid will contain Tet promoters, which the product of the Tet repressor gene will block the binding of DNA polymerase to. This plasmid would be used so transcription of the main cosmid genes would be blocked while packaging the cosmid. The main cosmid will be constructed out of the plasmid pUC18. A functioning cos site, which is the site of DNA packaging in phage, will be introduced to the plasmid, making it a “cosmid”. Using Gibson assembly, the Crispr cascade can be constructed with the Tet promoter that is to be block by the Tet repressor.

The modified Lambda phage, pCY1591, constructed by Cronan [8] will be utilized because it’s cos site is interrupted by a kanamycin resistance gene, disabling the phage’s ability to package its own DNA during cell lysis. Instead, when the lytic cycle is thermally induced, the cos site on the cosmid will be recognized, leading to the packaging of the Crispr cascade genes. The new cosmid-containing phage can then be collected and tested for proper packaging.

References

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