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Sequence-Specific Removal of Shiga Toxin-Producing Escherichia Coli Using the Crispr-Cas9 System

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Objectives

The CRISPR-Cas system (clustered regularly interspaced short palindromic repeats and CRISPR associated genes) has emerged as a programmable and versatile tool for precise genome editing. The specificity of the CRISPR-Cas9 system is dictated by a 20-nucleiotide CRISPR guide RNA. The Cas9 protein is a double-stranded DNA nuclease guided by guide RNA to sequence-specific sites. The interaction between the Cas9 protein and the target DNA sequences leads to lethal cleavage of double-stranded DNA. Objectives of this study were to design and clone a guide RNA targeting Shiga toxin in pCRISPR and use a 2-plasmid platform to deliver this Shiga toxin specific CRISPR-Cas9 system into bacterial cells for specific killing of Shiga toxin-producing *Escherichia coli.*

Materials and Methods

The *E. coli* O157:H7 cells containing pCRISPR were cultured on tryptic soy agar (TSA) with 50 µg/ml of kanamycin, cells containing pCas9 were cultured on TSA with 50 µg/ml of chloramphenicol, and those containing both pCRISPR and pCas9 plasmids were cultured on TSA with 50 µg/ml of kanamycin and 50 µg/ml of chloramphenicol. Shiga toxin guide RNA was designed by screening Shiga toxin gene sequences for NGG on the 3' side. The pCRISPR vector was digested with BsaI (10 units) and purified using a Monarch gel extraction kit (New England Biolabs). The designed guide RNA was ligated with the digested pCRISPR to create a new plasmid of pCRISPR w/stx. After ligation, the cloned region was sequenced by a Sanger Sequencing service (Genewiz).

The pCas9 and pCRISPR w/stx were introduced into *E. coli* O157:H7 cells sequentially by electroporation: first the pCas9 was introduced into *E. coli* O157:H7 cells and then the pCRISPR w/stx was introduced into the recipient *E. coli* O157:H7 cells containing pCas9 plasmids. Briefly, *E. coli* O157:H7 cells were grown to a A_{600} of 0.4 to 0.6. Plasmids (around 100 ng each) were transformed into bacterial cells using 0.1 cm Gene Pulser cuvettes (Bio-Rad) and the Electroporator 2510 (Eppendorf) set at 1800 V. In addition to pCRISPR w/stx, pCRISPR cloned with a piece of oligo that does not target any DNA sequences in *E. coli* O157:H7 cells (pCRISPR w/oligo) and the original pCRISPR were used as controls. After electroporation, cells were plated onto TSA plates containing appropriate antibiotics for quantification.

Results

Sanger sequencing confirmed that the guide RNA targeting Shiga toxin genes was successfully cloned in pCRISPR. When the newly created pCRISPR w/stx plasmid was introduced into the recipient *E. coli* O157:H7 cells containing pCas9 plasmids, an approximately 2 log lower concentration of *E. coli* O157:H7 cells was observed compared to that of the control plasmids of pCRISPR w/oligo and pCRISPR.

Conclusion

This study provides proof-of-concept evidence that introduction of a CRISPR-Cas9 system that specifically cleaves Shiga toxin genes in bacterial cells leads to death of Shiga toxin-producing *E. coli*. The CRISPR-Cas system could be further explored for improving meat safety by sequence-specific removal of pathogens that harbor target virulence or antibiotic resistance genes.

