Identification of Novel Chemical Compounds that Control *Escherichia coli* O157:H7 Through Use of a High-throughput Small Molecule Screen

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**Abstract**

*Escherichia coli* O157:H7 infections are responsible for a significant number of illnesses and fatalities caused by known foodborne pathogens each year in the United States. *E. coli* O157:H7 colonizes the gastrointestinal tract of healthy cattle and is thus commonly shed in their feces. As a result, contamination of beef by *E. coli* O157:H7 during slaughter is inevitable and reducing pathogen loads in beef remains a significant challenge for the beef industry. Although years of research led to the identification of a variety of interventions that are currently in-place to control *E. coli* O157:H7 contamination in beef, the national health objectives outlined to reduce the incidence of disease attributed to *E. coli* O157:H7 have not yet been met and a clear need exists to identify novel antimicrobials to enhance current mitigation strategies. Small molecules (typically < 500 daltons) have been useful to probe biological functions at the molecular and cellular levels as well as for treating disease and most therapeutic drugs fall within this category of molecules. We screened more than 64,000 small molecules (i.e., compounds from known bioactive and commercial libraries) for bactericidal activity against *E. coli* O157:H7 and 46 small molecules demonstrated bactericidal activity against a natural *E. coli* O157:H7 strain from cattle feces. Forty-three of these compounds are from known bioactive libraries and the other three compounds are from commercial libraries. Known bioactive compounds that inhibited *E. coli* O157:H7 growth predominantly included antimicrobials that have commonly been used as clinical interventions; however, two of these known bioactive antimicrobials have been used in non-clinical applications. Further investigation revealed that these two known bioactive antimicrobials damage bacterial outer cell layers and alter membrane permeability, suggesting that they will be effective in controlling a broad spectrum of pathogens. Potential applications for these known bioactive antimicrobials along with the three compounds from commercial libraries to reduce pathogen populations in beef have not been investigated. The utility of the small molecules identified here to inhibit *E. coli* O157:H7 growth will be evaluated in future studies as dipping and spray-washing treatments to reduce *E. coli* O157:H7 populations on cattle hides and beef tissues.

**Introduction**

*Escherichia coli* O157:H7 is a pathogen which causes roughly 62,000 illnesses, 1800 hospitalizations, and 52 deaths a year in the United States due to foodborne exposures (Mead et al. 1999).

*E. coli* O157:H7 can result in a serious disease called hemolytic uremic syndrome (HUS) which can result in kidney failure and death in susceptible populations (Karmali et al. 1985).

Cattle serve as a major reservoir of *E. coli* O157:H7 and contamination of beef during slaughter by this pathogen presents a major challenge to the beef industry (Blanco, 1996).

Here we implemented a high throughput small molecule screen in order to discover novel antimicrobials for use as interventions in the food supply or therapeutically in a clinical setting.

**Methods**

384 well plates were filled with Brain Heart Infusion Broth and *E. coli* O157:H7.

Each individual well is a separate reaction in which individual compounds can be tested.

**Results**

"Percent Inhibition" values were calculated by the following equation:

\[
\text{% inhibition} = \frac{\text{O.D.}_{600} \text{ of positive control} - \text{O.D.}_{600} \text{ of experimental well}}{\text{O.D.}_{600} \text{ of positive control}} \times 100
\]

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O.D. of positive control

O.D. of experimental well
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Absorbance readings were taken in a plate reader at 600nm.

**Conclusions**

- Here we identified 46 unique compounds which inhibit the growth of *E. coli* O157:H7.
- Two of these compounds are known bioactives, used in varying non-clinical applications.
- These compounds will be evaluated for further utility as spray-washing or dipping treatments to be used as interventions in the beef industry.

**Acknowledgements**


**References**

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