Exploring the Antimicrobial Properties of Honey Against the Bacterial Pathogen *Clostridioides difficile*

Karyme Lozano[1], Paola Zucchi[2], Ferhat Ozturk[1], and Jesús A. Romo[2]
[1]Department of Biology, Health, and the Environment
[2]Department of Molecular Microbiology and Immunology and The South Texas Center for Emerging Infectious Diseases
The University of Texas at San Antonio, San Antonio, TX

Abstract

Clostridioides difficile is a gastrointestinal bacterial pathogen able to take advantage of a dysbiotic microbiota environment to proliferate, secrete toxins, and damage the intestinal epithelium. *C. difficile* infection (CDI) dynamics are still not well understood, and a subset of patients will relapse after successful antibiotic (15-30%) or fecal microbiota transplant (FMT) (<10%) treatment. Here, we investigate honey's antimicrobial potential, particularly manuka honey, in combating epidemic strains of *C. difficile*. Using a 96-well plate assay, bacterial growth was monitored through optical density, comparing distinct honey samples to manuka honey controls. Results suggest honey, especially varieties with higher bioactive compounds, may serve as a natural alternative or complement to antibiotics in treating *C. difficile* infections.

Introduction

Honey presents a promising alternative in the fight against antibiotic resistance, particularly in addressing *Clostridioides difficile*, a bacterium that poses significant challenges in healthcare settings. *C. difficile* infections are common in patients whose gut microbiomes have been disrupted by antibiotics, and the bacterium's ability to produce spores and the development of resistance to commonly used antibiotics makes these infections difficult to treat. Honey, particularly manuka honey, is known for its antimicrobial properties, offering a potential solution to combat *C. difficile* infections. Manuka honey is especially valued for its high levels of methylglyoxal (MGO), which has demonstrated potent antibacterial effects. This research seeks to investigate the antimicrobial effects of various types of honey on *C. difficile*, with a focus on factors such as pH, moisture content, and antioxidant activity (measured by DPPH levels) that may influence its efficacy. By examining the antimicrobial properties of different honey samples, the study aims to highlight honey's potential as an effective treatment for *C. difficile* infections, particularly varieties with higher concentrations of bioactive compounds. It is anticipated that honey will inhibit the growth of *C. difficile*, offering a natural alternative or complement to traditional antibiotic treatments and contributing to the ongoing fight against antibiotic resistance.

Hypothesis and Aims

We have developed a 96-well plate assay to rapidly screen large numbers of honey samples against *C. difficile*.

We hypothesize that honey, specifically those with higher concentrations of bioactive compounds will display antimicrobial activity against *C. difficile*.

To test this hypothesis, we propose the following Aims:

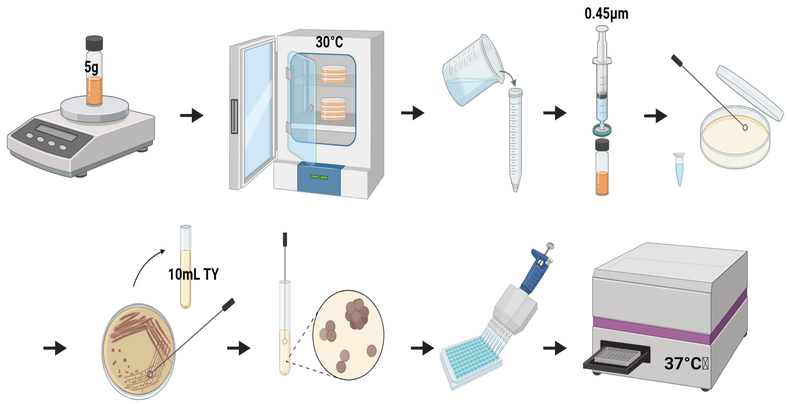
1. Test activity of honey at a fixed concentration against the epidemic UK1 strain of *C. difficile*.
2. Conduct dose response assays to determine minimal inhibitory concentrations (MICs) of active honey samples.

Strains and Methods

Table 1. *C. difficile* strains used

630Δ*erm*	Spontaneous erythromycin sensitive derivative of the reference strain 630 obtained by serial passaging in antibiotic free media.[1] Common strain used for genetic manipulation.
NAP1/B7/027-UK1	North American pulsed-field gel electrophoresis type 1, restriction endonuclease analysis type B1, polymerase chain reaction ribotype 027, which has shown a much higher recurrence rate than other strains.[2] Hypervirulent strain.

Strains and Methods Continued



Results

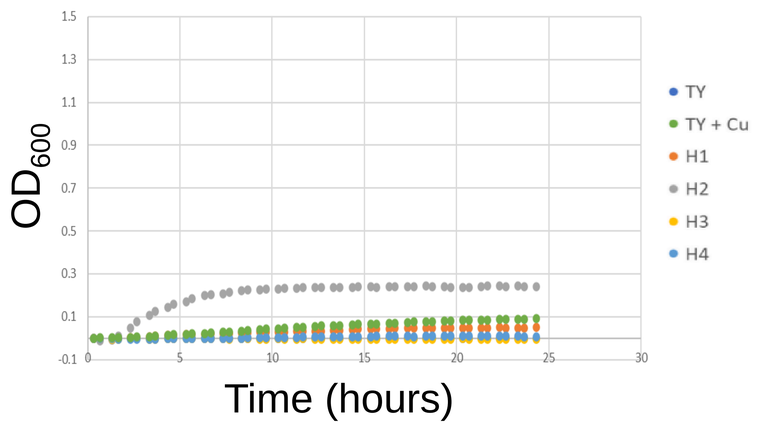


Figure 1. Filtration of honey samples removes impurities. Honey samples (H1-H4) were diluted in TY media (50% w/v), added to a 96 well plate and incubated in a plate reader overnight at 37 °C in the anaerobic chamber. Optical density (OD_{600}) was measured every 20 minutes. An increase in optical density was detected in the unfiltered sample H2 (**A**) suggesting a potential impurity or native microbial contamination. The impurity was removed after honey samples were passed through a 0.45 µm nylon filter (**B**). TY media and TY media with copper were used as controls.

Results

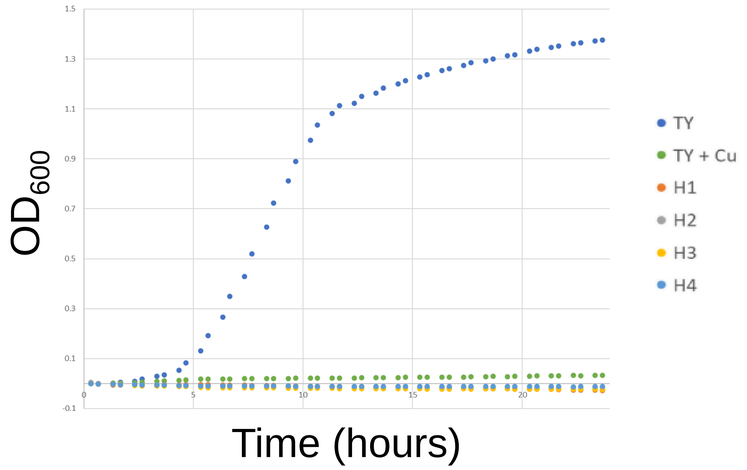


Figure 2. All honey samples tested display potent activity against *C. difficile*. Honey samples (H1-H4) were diluted in TY media (50% w/v), added to a 96 well plate containing a 1:100 dilution *C. difficile* UK1 from an overnight culture and incubated in a plate reader overnight at 37 °C in the anaerobic chamber. Optical density (OD_{600}) was measured every 20 minutes. All tested honey samples (H1-H4) displayed potent activity against *C. difficile*. Normal growth of C. difficile was observed in TY media alone. TY media with copper was used as a positive control as copper has antimicrobial activity against *C. difficile*.

Conclusions

- Filtration of honey samples does not reduce antimicrobial activity, but does remove native microorganisms and large particles
- All tested honey samples and the Manuka Honey control displayed potent activity against the epidemic UK1 strain of *C. difficile*.
- Honey hold therapeutic potential for the treatment of infections caused by *C. difficile*.

Future Directions

- Continue screening the honey library available at UTSA
- Conduct dose response assays to determine minimal inhibitory concentrations (MICs) of active honey samples.
- Test the most potent honey samples against *C. difficile* biofilms
- Determine which components of honey contain the anti-*C. difficile* activity

References

- JT;, L. D. (n.d.). *Clostridium difficile* infection. The New England journal of medicine. https://pubmed.ncbi.nlm.nih.gov/25875259/
- ES;, H. E. (n.d.). Antibacterial effect of manuka honey on *Clostridium difficile*. BMC research notes. https://pubmed.ncbi.nlm.nih.gov/23651562/

Acknowledgements

We would like to extend our sincere appreciation to the USDA Next Gen Program for their generous support and funding, which made this research possible. Additionally, we are deeply grateful to Dr. Paola Zucchi, Dr. Jesús Romo and Dr. Ferhat Ozturk for their invaluable guidance, expertise, and encouragement throughout this project.

This work was supported by start-up funds from The University of Texas at San Antonio (UTSA) to J.A.R. and the Honey Pathway Program to K.L.
The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the materials, and the content is solely the responsibility of the authors.