Determination of physical and chemical mechanisms to prevent *Cyclospora* infection

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Summary

The coccidian foodborne parasite *Cyclospora cayetanensis* is an emerging public health concern in North America. Previously, cyclosporiasis was associated with international travel to tropical or subtropical regions, but contaminated fresh produce, such as lettuce and cilantro, have fostered recent domestic outbreaks. Heat inactivation has proven effective but remains untenable for sterilizing fresh produce. Therefore, novel chemical or physical inactivation mechanisms suitable for implementation in this environment are desired.

We have developed an automated pipeline to evaluate potential antimicrobial compounds against coccidia. Combining robotics and machine learning, we can establish antimicrobial assays for several dozens of compounds in a few hours. Several initial candidate compounds are currently under evaluation. Additionally, a simple bulb/ballast scaffold for UV lamp sterilization is being constructed for laboratory validation.

Objectives

Robust molecular methods to assay the infectious potential of *Cyclospora cayetanensis* oocysts have not been developed. Instead, visual identification of sporulated (infectious) oocysts remains the gold standard. This process is both labor- and time-intensive and relies on the experience of a trained parasitologist. This bottleneck precludes high-throughput screening of candidate antimicrobial compounds.

For this project, we sought to develop a cost-effective, automated workflow, combining robotic assay assembly and image detection algorithms to evaluate the sporulation capacity (inactivation rate) of oocysts treated with several candidate compounds.

Methods

Serial dilutions of a list of compounds curated from the literature were tested for antimicrobial activity against surrogate coccidia species due to a scarcity of viable *C. cayetanensis* oocysts. We employed an Opentrons OT-2 liquid handling platform equipped with 8-channel pipet attachments to populate 96-well plates with candidate compounds dissolved in DMSO, as well as DMSO-only and distilled water non-treatment controls. A 96-well plate is prepared in approximately 5 minutes, with electronic pipets providing exquisite precision. Following incubation in potassium dichromate at 25°C for 10–14 days, oocysts are imaged via confocal microscopy. These image files are then used as input for a detection algorithm trained to recognize and enumerate sporulated oocysts, facilitating automated evaluation of inactivation potential. The resulting counts are compared against a trained parasitologist's evaluation of the same specimens, and the level of agreement is provided as a metric of the AI efficacy.

Results to Date

Our robotic platform facilitates assembly of dozens of chemical inactivation assays in a matter of hours (**Figure 1**). Encapsulated and free polyphenol (cucurmin, quercetin, and luteolin), ellagic acid, resveratrol, thymol, epicatechin, and carvacrol assays are currently underway, challenging a heterogenous suspension of *Eimeria* spp. obtained from a poultry research facility. This stock shows sporulation of untreated oocysts (suspended in 2.5% potassium dichromate) at 30% after 5 days, vs. 13% in the stock suspension (**Figure 2**). Quantitative demonstration of antimicrobial activity for these compounds is anticipated in 1–2 weeks.

We continue to train our image detection algorithm with tens of thousands of annotated images of sporulated oocysts. Each image is accompanied by the x, y, height and width coordinates measured in pixels (**Figure 3**).

Benefits to the Industry

We anticipate that this work will result in the identification of several methods for inactivating *C. cayetanensis* and related coccidia, which will be suitable for implementation in the appropriate settings, either for purifying contaminated water sources or sterilizing fresh produce. We continue to expand the panel of compounds to be tested. Furthermore, by obviating the need for laborious microscopic examination of oocysts, we propose that our image detection algorithm will be a useful tool for the research community and reduce the barrier for entry into work toward discovering mechanisms for *C. cayetanensis* inactivation.

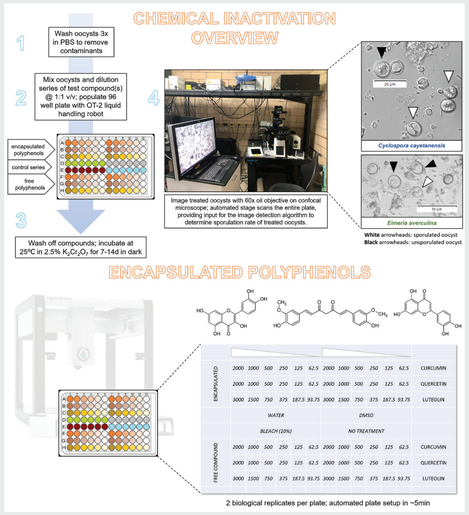


Figure 1. Workflow overview of chemical inactivation assays. Robotics and machine learning facilitate high-throughput enumeration of sporulated vs. nonsporulated oocysts following chemical administration.

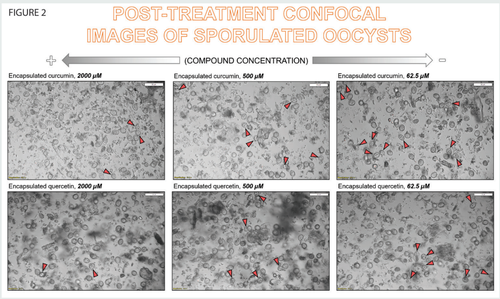
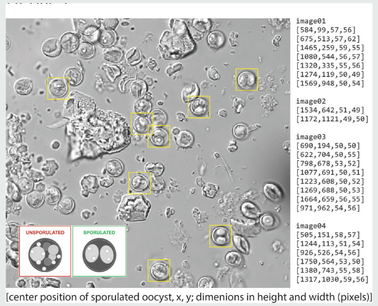


Figure 2. Examples of micrographs obtained via confocal microscopy of an initial round of compound testing. Sporulated oocysts are indicated with red arrowheads.



[center position of sporulated oocyst, x, y; dimenions in height and width (pixels)]

Figure 3. Machine learning is used to detect sporulated oocysts, facilitating rapid, automated enumeration of viable oocysts as a readout for each chemical compound's antimicrobial capability, or physical disruption of oocysts (UV). Shown is a representative image used for training the AI. Sporulated oocysts are annotated with x and y coordinates (in pixels), followed by height and width measurements in pixels.