Cyclospora cayetanensis monitoring in agricultural water

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Summary

Cyclospora cayetanensis causes illness in people consuming infected produce. Because this pathogen is in very low concentrations in fresh produce, which makes difficult to detect, and for prevention reasons, it is more effective to check for its presence in irrigation water, from where it is typically transferred onto produce. However, even in water, this parasite is very difficult to detect as there is no antibody or other recognition molecule that would bind with this parasite. We are working on designing and synthesizing, for the first time, aptamers that will bind to intact *Cyclospora cayetanensis* oocysts, and use them to design simple paper-based colorimetric tests that can detect it in the field without the need for sample preparation or specialized laboratories.

Objectives

1. Design and understand the parameters for the *Cyclospora cayetanensis* aptamer synthesis via SELEX – systematic evolution of ligands by exponential enrichment.
2. Design and test a colorimetric microfluidic biosensor platform for the detection of *C. cayetanensis* intact oocysts spiked in agricultural water (from irrigation ponds around Purdue) and measure parameters critical for achieving biosensing functionality (including multi-replicate ability, selectivity, repeatability, response time, detection limits, and stability).

Methods

Obtaining *Cyclospora* spp.–positive samples to perform the positive SELEX round is difficult. Hence, we have identified and expressed *C. cayetanensis* oocyst protein TA4. Purified recombinant protein is being used in the aptamer selection process. A DNA library with ≥10^{15} unique sequences has been designed, and PCR amplified for SELEX. The amplified DNA library was purified using PCR gel purification kit. Positive selection rounds allow binding of the DNA library to the recombinant proteins (**Figure 1**). The bound DNA is eluted for use in the counter selection round with *Eimeria* spp., to select highly specific aptamers with minimum cross-reactivity.

A device named PURE-SCAN (Purdue's Enhanced Sensing System for Cyclospora Analysis in Agricultural Water using Nanomaterials) was designed by the team for on-site analysis of microfluidic paper-based analytical device (μPAD) sensors.

Results to Date

This study reports the overexpression and purification of the *C. cayetanensis* specific protein TA4 for the selection of aptamers. To date, we have performed 10 SELEX rounds. During these rounds, the ssDNA library or the last eluent from the previous SELEX round was preincubated with pristine magnetic beads for negative selection (**Figure 2**), or with *Eimeria tenella* or *E. maxima* oocysts for counter selection rounds (**Figures 3** and **4**). For positive selection rounds, the ssDNA library or last eluent from the previous SELEX round was incubated with TA4-conjugated magnetic beads (**Figure 1**).

The PURE-SCAN device (**Figure 5**) is designed for on-site analysis of paper-based μPAD sensors and is being tested at the lab scale to record and analyze the colorimetric sensor response in the presence and absence of *C. cayetanensis* proteins in the test sample.

Benefits to the Industry

Demonstrating Cyclospora cayetanensis detection in a multiplexed and multi-replicate fashion will offer a solution to efforts to reduce the number of illness outbreaks due to contaminated produce.

Our paper-based device is based on a novel integration of existing detection technologies, and if successful, will provide regulatory agencies and businesses with compelling monitoring capabilities of agricultural water.

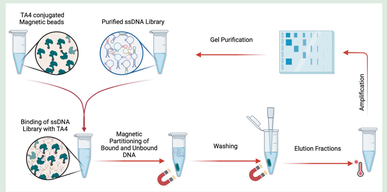

Figure 1. **Positive SELEX strategy** employed to select an aptamer that specifically binds to the TA4 antigen of *Cyclospora cayetanensis*. Figure illustrates the process of incubating the ssDNA library or the last eluent from the previous SELEX round with TA4 conjugated magnetic beads. The unbound oligonucleotides were removed, and the bound oligonucleotides were eluted from the beads. The eluted oligonucleotides were amplified through preparative PCR to generate the next round's ssDNA library. This process was repeated for several rounds, enriching for aptamers that specifically bound to the TA4 antigen of *C. cayetanensis*.

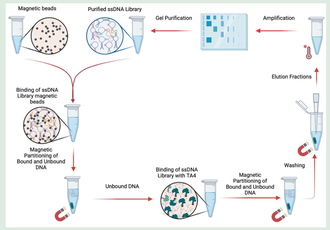

Figure 2. **Negative SELEX strategy** employed to select an aptamer that specifically binds to the TA4 antigen of *C. cayetanensis*. Figure depicts the process of preincubating the ssDNA library or the last eluent from the previous SELEX round with pristine magnetic beads to remove any nonspecific binders. This step ensured that only ssDNA molecules that did not bind to the pristine magnetic beads were used for the subsequent positive selection rounds.

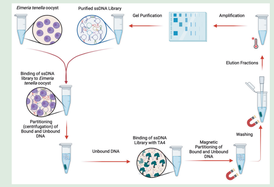

Figure 3. **Counter SELEX strategy** employed to select an aptamer that specifically binds to the TA4 antigen of *C. cayetanensis*. Figure illustrates the process of preincubating the ssDNA library or the last eluent from the previous SELEX round with *Eimeria* spp. oocysts to remove any aptamers that cross-reacted with the *E. tenella* oocysts. The unbound ssDNA molecules were then used for subsequent positive selection rounds with TA4 conjugated magnetic beads. Through this approach, aptamers that specifically bound to the TA4 antigen of *C. cayetanensis* and did not cross-react with the *E. tenella* oocysts were enriched.

Figure 4. Gel electrophoresis image of the PCR amplified eluates obtained during the 10th round of the SELEX process.

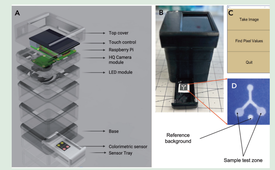

Figure 5. Schematic (A) of the 3D printed **PURE-SCAN device** (B) developed for detecting *Cyclospora cayetanensis*. The device consists of a Raspberry Pi 4B and a high-quality camera system with LED lights. The paper-based colorimetric sensors used in the device change their native red/reddish-pink color (analyte absent) to blue/blueish-gray in the presence of C. cayetanensis in the test sample. The device takes an optimally illuminated image of the μPAD (D) colorimetric sensors and processes it to create a calibration curve for quantitatively determining the concentration of contaminating pathogen(s) in the test samples. The program responsible for image analysis is written in Python and contains two main functions, accessible by graphical user interface (C) buttons.