Cyclospora cayetanensis monitoring in agricultural water

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Summary

Cyclospora cayetanensis causes illness in people consuming infected produce. This pathogen is found in very low concentrations on actual produce, which makes it close to impossible to detect. Also, for prevention reasons, it is more effective to check for *Cyclospora* 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.

In this project, we are working to design and synthesize, for the first time, aptamers that will bind to intact *Cyclospora cayetanensis oocysts*, and use these aptamers to design simple paper-based colorimetric tests for rapid in-field detection of oocysts without the need for extensive 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 detection of *C. cayetanensis* intact oocysts spiked in agricultural water, and measure parameters critical for achieving biosensing functionality.

Methods

Work to date has focused on Objective 1. Obtaining *Cyclospora* spp.–positive samples to perform the positive SELEX round is difficult. Hence, we are in the process of expression and purification of *C. cayetanensis* oocyst proteins (**Table 1**). The purified recombinant proteins will be 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 the QIAEX II gel purification kit. Positive selection rounds allow binding of the DNA library to the recombinant proteins. The bound DNA will be eluted for use in the negative selection round with *Eimeria acervulina*, to select highly specific aptamers with minimum cross-reactivity.

Results to Date

Design and preparation of DNA library

The DNA library being used in the present study (5'-ATCCGTCACACCTGCTCT-N36-TGGTGTTGGCTCCCG TAT-3') has a mean melting temperature of 72.8°C. Amplification of the DNA library without any cross-product was obtained at an annealing temperature of 55°C, 1.75 mM Mg^{2+}, and template-to-primer ratio of 100 ng:1 µM, as shown in **Figure 1**.

Cloning and expression of *C. cayetanensis*–specific proteins in *E. coli*

We are targeting five proteins as potential biomarkers for *Cyclospora* spp. These proteins are known to be found in the Cyclospora spp. oocyst. We are in the process of isolating these recombinant *Cyclospora*-specific proteins (**Table 1**). These proteins will be used as positive control targets during SELEX and the subsequent assay development stage. The proposed SELEX strategy is depicted in **Figure 2**.

Benefits to the Industry

Demonstrating *Cyclospora cayetanensis* detection in a multiplexed and multi-replicate fashion will offer a solution to the effort of lowering the numbers of 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 for agricultural water.

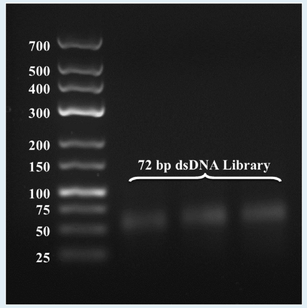

Figure 1. Amplification of the DNA library to obtain a 72 bp dsDNA amplicon to be used during SELEX. Amplification of the DNA library without any cross-product was obtained at an annealing temperature of 55°C, 1.75 mM Mg2+, and template-to-primer ratio of 100 ng:1 µM.

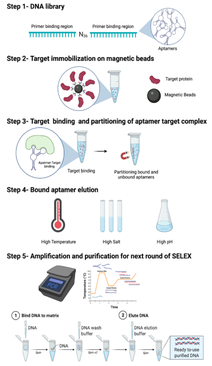

Figure 2. *Cyclospora cayetanensis*–specific aptamer selection strategy being employed in this project.

Table 1. *Cyclospora cayetanensis* protein biomarkers being used for selection of aptamers

1.	Sporulated oocyst TA4 antigen
2.	Oocyst wall protein 2
3.	Heat shock protein 70
4.	Cell wall protein RBR3
5.	Vegetative cell wall protein gp1