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Simple and fast detection of E. coli in agricultural water sources and runoff

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Disciplines
Agriculture | Biology | Environmental Public Health | Public Health

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Budget:
$20,000 for year one

Q 1) Can T4 phage be stabilized on paper? and
2) Can *E. coli* be detected with an assay stored on paper?

ECOLOGY

A The first question was answered by applying and drying T4 phage on paper and exposing the phage to different potentially harmful conditions. The second question was answered by adding and drying an assay for malate dehydrogenase on paper followed by the addition of *E. coli* in liquid. Positive results for the first question were active phage after drying or exposure and to the second question was a color change in the presence of bacteria.

Background

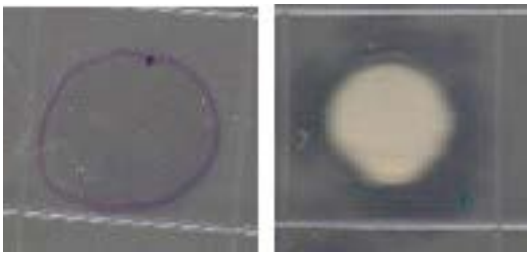
Harmful microorganisms are the leading cause of water quality impairments in the United States, and are thought to be responsible for 900,000 illnesses and 900 deaths per year. Pathogens originate from many different sources, among them agricultural operations such as land application of animal manure or allowing cattle to have direct access to streams. Other outlets include human sources such as leaking septic systems and wildlife sources such as migratory birds. While total prevention of water contamination is impossible, quickly and accurately detecting the presence of potentially pathogenic microorganisms is important to protect public health.

One critical indicator of pathogen-contaminated water is *Escherichia coli (E.coli)*. These bacteria are typically detected by taking a water sample and filtering it to capture the bacteria on the filter paper. That filter paper is placed on nutrient-enriched agar and the bacteria are allowed to grow under controlled conditions for 24 hours, after which the number of bacteria colonies is counted. This method, while accurate, is time-intensive (one to two days) and must be done in a laboratory. The risk from contaminated water could be significantly decreased with the use of rapid *E. coli* detection kits.

These quick and accurate detection techniques are badly needed to better identify waters posing a risk to animal and human health. Investigators believed they might be able to use bacteriophages—naturally occurring viruses that attack and potentially destroy bacteria—to improve detection techniques. The objective of this study was to generate preliminary data in two important areas:

- stability of bacteriophages on paper, and
- development of a sensitive colorimetric assay for bacteria on paper;

for the development of a quick and easy-to-use paper-based test for the detection of bacteria like *E. coli* in agricultural waters and runoff.



Activity of T4 phage after exposure to pH11 for 5 minutes in solution (left) and 10 hours on paper (right). The paper has a diameter of 6 mm and the purple ring around the paper shows the extent of the clearing due to phage activity. There was no activity of phage at any later time points tested.

Approach and methods

One culture-based technique for the detection of bacteria is phage-typing. This technique uses bacteriophages, viruses infecting bacteria, and is very specific in the detection of bacteria. It requires one to two days before results are available. During the last few years, researchers have started to use bacteriophages as recognition elements in faster detection techniques by binding them to material surfaces.

To achieve the objectives of the study, the researchers conducted two separate areas of investigation.

1. To test the stability of bacteriophages on paper, they added bacteriophages to filter paper and allowed the paper to dry. They then stored the dry paper to test the shelf life. They also tested the influence of high pH and temperature on the stability of the phage in solution and on paper.

2. To prepare a colorimetric assay on paper, they determined the most common enzymes in *E. coli* and their colorimetric substrates. They then tested different ratios of the assay components to determine the lowest number of bacteria that could be detected on paper.

Results and discussion

The research team was able to attach bacteriophages for *E. coli* to filter paper. After drying the paper to allow for easier storage, they could retain the paper with the bacteriophages for at least one month and still have active phage on the paper. They used the active phage on these papers to capture specific bacteria from the water sample they chose to test.

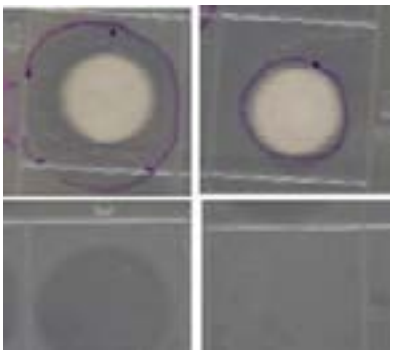
Investigators tested the stability of the bacteriophages to harsh conditions and found that at a pH of 10, the bacteriophage on the paper were active for twice as long compared to the same bacteriophage in water. When the temperature was increased to 70°C, they saw no difference in the stability of the bacteriophage on paper compared to those in solution and all phage were deactivated after 15 minutes of exposure.

To develop a colorimetric assay for *E. coli* on paper, they added chemicals to the paper that would turn color in the presence of live bacteria. When they applied bacteria to the paper containing the chemicals needed for the assay, there was a change in color from light yellow to red in a matter of two hours. This detection time is much shorter than the current time needed to detect bacteria from water samples. However, at this point they were not able to detect low enough numbers of bacteria to make the assay viable to use in the field.

Conclusions

The major findings for this research project are:

1. bacteriophages can be dried on paper and stored for up to one month;
2. paper helps stabilize phage against elevated pH,
3. paper does not improve survival of phage at elevated temperatures; and
4. bacteria can be detected on paper using tetrazolium salts with a limit of detection of 104 CFU/paper.



Activity of T4 phage after the exposure to 70C on paper (top row) and in solution (bottom row) for 5 minutes (left) and 15 minutes (right). The paper has a diameter of 6 mm and the purple ring around the paper shows the extent of the clearing due to phage activity.

While all this information is important for the development of a dip-stick test for bacteria in water, these results alone are not sufficient. Further work needs to be done to extend the storage time to at least six months and further test environmental conditions found in agricultural waters. For the assay, testers have yet to lower the limit of detection from 10,000 CFU/paper to 10 or 100 CFU/paper to be able to determine bacteria concentrations in an environmentally relevant range.

Researchers have to combine the phage on the paper with the assay to concentrate the bacteria of interest on the paper before detecting them. Once the necessary laboratory test has been performed, the results can be transferred to the field. While there is still much work remaining, the investigators were able to generate crucial preliminary data in this study, which will make future grant proposals to other funding agencies more competitive.

Impact of results

Stability of bacteriophages on paper

The researchers were able to stabilize bacteriophages on paper and store them for extended periods of time. They were impressed at how well the bacteriophages were stabilized, especially given a change in pH and the removal of moisture.

Design of a colorimetric assay for bacteria on paper

The researchers were able to detect bacteria on paper using tetrazolium salts, but the limit of detection is too high to be environmentally relevant. In the future, they will need to either pre-concentrate or amplify the bacteria before detection or they will need to improve the assay further, e.g., by changing the size of the detection zone or by adding substrates for other enzymes to have two or more colors change simultaneously.

Once they have united these components and added the relevant steps to improve the limit of detection, Iowa farmers will be able to test their water for contamination with *E. coli.* Knowing the levels of *E. coli* could allow the farmers to decide if they should use this water for irrigation or animal production.

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Education and outreach

Two publications are in progress. Because the study was intended to gather preliminary data, the results have not been reported to farmers or the public. Sharing of data would occur after a working test is available.

No graduate students participated in the project, but seven undergraduates in chemical engineering, microbiology and biochemistry worked on the project.

Leveraged funds

No additional funds were leveraged by this project.