

# The Use of Plasmid DNA from *E. coli* to Determine the Relative Frequency of

## Plasmids in Bacteria of Lake Michigan

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### Abstract

The contamination of the world's water sources has become a major problem. Lake Michigan, a recreational water source, has become vastly polluted primarily by antibiotic resistant strains of *E. coli*. In the project hypothesis that there are varied populations of plasmids in the enteric bacteria of Lake Michigan was tested. A scheme was developed that utilized plasmid probes to evaluate bacteria from Lake Michigan water samples and determine the relative frequency of each type of plasmid. A probe, synthesized from SS 6.25 Mac B DNA, proved to show specificity when tested with other DNA samples. During colony hybridization the Mac B probe was found to not be specific enough. A new probe was synthesized from *TaqI* cut SS 6.25 Mac B DNA and used in colony hybridization. The implications of the results that were achieved in this experiment show that a viable method was developed to probe water samples for the presence of plasmids and allow for the relative plasmid population frequency to be determined.

### Introduction

- Many different nonpoint sources have been identified such as domestic and wild animal defecation, sewage overflows, urban stormwater, farm waste runoff, and industrial wastes
- In order to protect public health the use of indicator organisms, such as *E. coli* or fecal coliforms, has been employed
- *E. coli* colonizes the gut of warm-blooded animals, it is not normally pathogenic, easy to culture and detect, and it is present in water in much higher concentrations than other bacteria – which makes *E. coli* a common choice for indication of fecal contamination
- Previous studies have demonstrated a direct correlation between the density of *E. coli* in water and the occurrence of gastroenteritis as a result of swimming
- Water sources used in this study were collected from Milwaukee area beaches along Lake Michigan (South Shore Beach (SS), Bradford North (BN), and Bradford South (BS))
- Gel electrophoresis was used to characterize the plasmid DNA and Southern Blot analysis was used to determine the frequency of particular plasmids in the different samples

### Hypothesis

The results from the study will show that the method formulated in this particular case will provide insight regarding the relative frequency of antibiotic resistant genes in water batch samples.

### Methods

#### Gel electrophoresis:

The *E. coli* samples isolated from Lake Michigan were characterized using this method. The bands of the DNA migrated based upon their corresponding size. They were characterized in conjunction with *HindIII* cut DNA. The gels were run at 80V for 60 min. Pictures were taken of each gel and kept for verification of results.

#### Southern Blot:

Hybond nitrocellulose was used in the transfer of DNA from the agarose gel to be used for SB analysis. Labeled DNA probes were utilized to detect the presence of particular DNA specified for in certain trials. ECL Direct Nucleic Acid Labeling and Detection System from Amersham Biosciences was employed for probe labeling and DNA detection on film.

### Results

- 31 different *E. coli* DNA samples were characterized according to plasmid DNA size and migration in agarose gel electrophoresis
- Lambda DNA, the control was then used to begin the experimental process

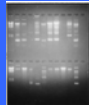


Figure 1: Agarose Gel Electrophoresis of Plasmids. All samples isolated from water were separated on agarose gels to see the size. Lambda DNA cut with *HindIII* is used as a migration standard in the last lane on the left.

- The Southern blot control trial, which utilized *HindIII* cut Lambda DNA as the probe, showed specific binding event by film exposure bands found solely where *HindIII*  $\lambda$  present

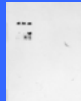
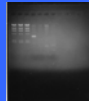


Figure 2b: Southern Blot film from the DNA transfer from the control gel pictured in Fig. 2a. The observed bands show the areas in which the lambda probe bound to the Lambda DNA.

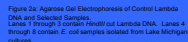


Figure 2a: Agarose Gel Electrophoresis of Control Lambda DNA and Selected Samples. Lanes 1 through 3 contain *HindIII* cut Lambda DNA. Lanes 4 through 6 contain *E. coli* samples isolated from Lake Michigan cultures

- SS 6.25 Mac B plasmid was chosen as a trial probe synthesized from a small 2kb segment
- DNA present in this trial included *HindIII*  $\lambda$  SS 6.25 Mac B DNA, and SS 6.25 Mac C DNA
- The Southern Blot film shows that the SS 6.25 Mac B probe was specific enough to solely bind to the SS 6.25 Mac B DNA from which it was made.

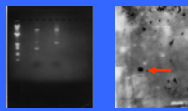


Figure 3b: Southern Blot film of the agarose gel pictured in Fig. 3a. The dark band shown in the above picture verifies that the SS 6.25 Mac B probe bound solely and specifically to the 2kb segment of the SS 6.25 Mac B DNA from which it was made.



Figure 3a: Agarose Gel Electrophoresis of *HindIII* cut Lambda DNA, SS 6.25 Mac B DNA, and SS 6.25 C DNA. The small 2kb segment of the middle sample, SS 6.25 Mac B DNA is the one to which the SS 6.25 Mac B probe will bind.

- The SS 6.25 Mac B probe was tested in a colony hybridization
- The film from the Southern Blot showed extensive binding to every colony present
- Due to these results it was determined that the SS 6.25 Mac B probe needed to be made more specific



Figure 4: Southern Blot film of the colony hybridization while using the SS 6.25 Mac B probe. The film shows extensive binding of the probe to the DNA found on the membrane. Due to the nonspecific binding the probe needs to be made more specific.

- The probe was made more specific by using restriction enzymes to cut the DNA
- *TaqI* cut SS 6.25 Mac B was made into a probe.

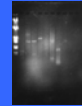


Figure 5: Agarose Gel Electrophoresis of *HindIII* cut Lambda DNA and seven lanes from the left containing the cut performed by *TaqI* that was subsequently purified and used as the next trial. The portion used to make the probe in the 1000bp segment located in the lower area of the lane.

### Discussion

- The results of Southern blot demonstrate that the SS 6.25 Mac B probe was specific since it only bound to the DNA from which it was synthesized
- Results of the colony hybridization showed extensive binding of the probe to every colony
- Probe was made more specific by cutting it with restriction enzymes
- The S<sub>6B</sub> segment that was formed by a *TaqI* cut was purified and used as the next probe
- Results showed a single band that formed in the (*TaqI*) SS 6.25 Mac B DNA lane
- Further research needed to make probe more stringent so binding occurs in all lanes since they are all Mac B
- The implications of the results that were achieved in this experiment show that a viable method was developed to probe water samples for the presence of plasmids and allow for the relative plasmid population frequency to be determined
- This aspect is of importance because it contributes to the survivability of the host organism which in this case is *E. coli* as a pollutant of Wisconsin's recreational waters.

### References

- McLellan, Sandra L., Annette D. Daniels, and Alissa K. Salmore, "Genetic Characterization of *Escherichia coli* Populations from Host Sources of Fecal Pollution by Using DNA Fingerprinting," *Applied and Environmental Microbiology*, May 2003, 2587-2594.
- McLellan, Sandra L., Annette D. Daniels, and Alissa K. Salmore, "Clonal Populations of Thermotolerant Enterobacteriaceae in Recreational Water and Their Potential Interference with Fecal *Escherichia coli* Counts," *Applied and Environmental Microbiology*, Oct 2001, 4934-4938.

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