Comparison of Agar and Pectin Based Methods for the Detection of Male Specific Coliphage

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Abstract

Water quality is an important public health concern affecting millions of lives. Many waterborne illnesses are caused by enteric virus contamination in the water. Current test methods involve the detection of bacterial indicators which is inadequate in predicting the pathogenic levels of enteric virus. Due to similarities in structure, composition, and resistance to environmental extremes as enteric virus, coliphages have been considered as a surrogate for determining enteric virus levels in water quality assessment. This study focused on the detection of male specific (F+) coliphage using agar based EPA Method 1602, single agar layer (SAL) in comparison to commercially available pectin based Easyphage. Both methods involved combining surface water, sewage, or spiked samples with log-phase *Escherichia coli* to either media. After appropriate incubation, detection of coliphage was identified by plaque formation in a lawn of *E. coli* host growth. In spiking experiments, samples with a pre-determined concentration of F+ coliphage corresponding to 80 plaque forming units (PFU) per 100mL were tested by both methods to determine recovery rates. In spiked samples, the highest recovery rate for SAL was 25% (20/80) and 98% (78/80) for Easyphage with a mean recovery rate of 7% and 69% respectively. In surface water samples, Easyphage consistently showed more recovery of coliphage than SAL method with a mean recovery of 123.6 PFU/100ml for Easyphage and 53.8 PFU/100ml for SAL. In one sample, Easyphage recovered 25 times more coliphage than SAL. The pectin based Easyphage method is less labor intensive and more effective in the recovery of F+ coliphage in comparison to the agar based SAL method. Incorporating the Easyphage method along with traditional bacterial indicators would provide a more complete assessment of water quality.

Materials and Methods

- 20 surface waters, 2 sewage samples, and 25 samples spiked with 80 PFU/100mL were tested using agar based EPA Method 1602, single agar layer (SAL) in comparison to a commercially available pectin based media, Easyphage 100 (Scientific Methods, Granger, IN).
- Male specific (F+) MS2 coliphage (ATCC#15597-B1) was used in conjunction with log-phase *E. coli* Famp host (ATCC#700891). *E. coli* host must be in log-phage to express F+ pili for F+ coliphage attachment and host infection to occur resulting in plaque formation (Fig. 1-2).
- 100X Ampicillin sodium salt and streptomycin sulfate (Sigma Aldrich, St. Louis, MO) were added to surface water and sewage samples to prevent high background bacteria from interfering with coliphage recovery.
- Refer to EPA Method 1602 for details on materials and methods for the SAL and double agar layer (DAL) method. In spiking experiments, DAL was used to enumerate stock suspensions of F+ coliphage to determine a spike dose of 80 PFU per 100mL of trypicase soy broth (TSB) (Becton Dickinson, Franklin Lakes, NJ). Clear circular zones in a bacterial lawn were counted as plaques and are expressed as PFU/ 100mL (Fig. 4). www.epa.gov/microbes/1602ap01.pdf
- Easyphage procedure involved the addition of 2mL of antibiotics, 0.7mL of bacterial stain, 3.5mL of log phase *E. coli* host, and 100mL of water sample to the Easyphage media (Fig. 3). The solution was mixed and 20mL was dispensed into each calcium coated petri plates for a total of 10 plates. Easyphage is a pectin based medium that reacts with the calcium coated petri plates causing the media to solidify. The media either solidified on the bench for 1hour or was incubated immediately upright at 36˚C ± 1˚C for 16-24hr. Blue plaques in a red bacterial lawn were counted on all 10 plates and expressed as PFU/100mL (Fig. 5).
- Each run included TSB as a blank control and a spiked sample as a positive control.

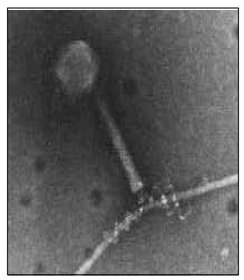


Figure 1. Phage attachment
Courtesy of ASM, Merry

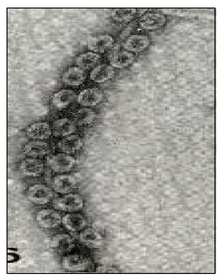


Figure 2. MS2 coliphage attachment to F pilus
Courtesy of emergentcomputation.com

Figure 3. Easyphage 100 media, bacterial stain, & calcium coated plates

Plaque Formation

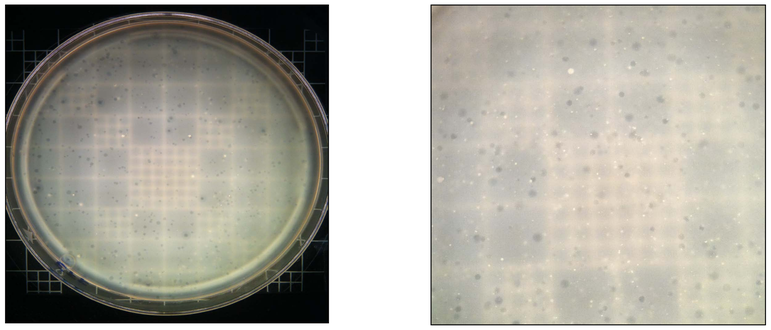


Figure 4. Agar based Single Agar Layer (SAL) clear zone plaques

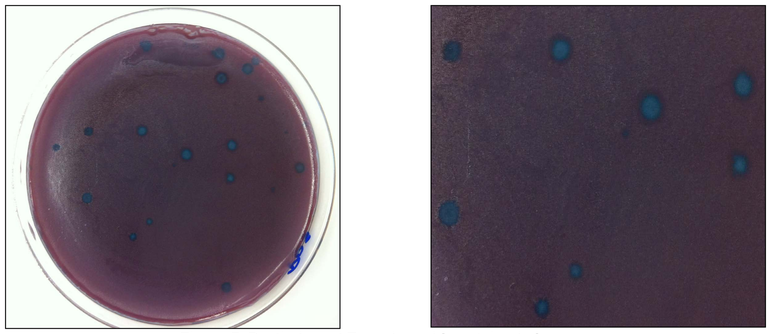


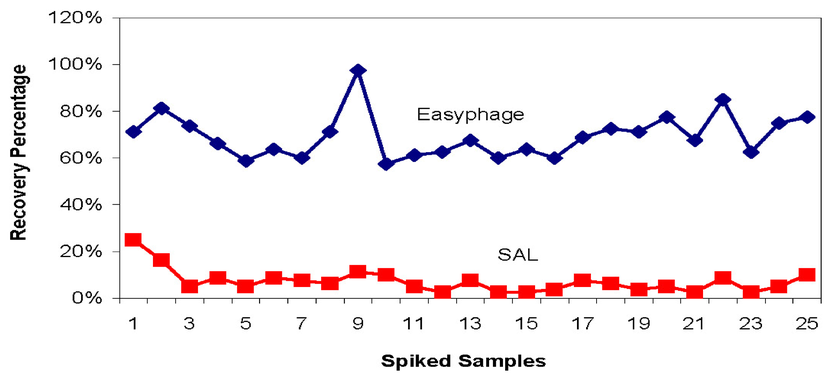
Figure 5. Pectin based Easyphage blue stained plaques

Results

Table 1. Single agar layer and Easyphage Recovery Summary

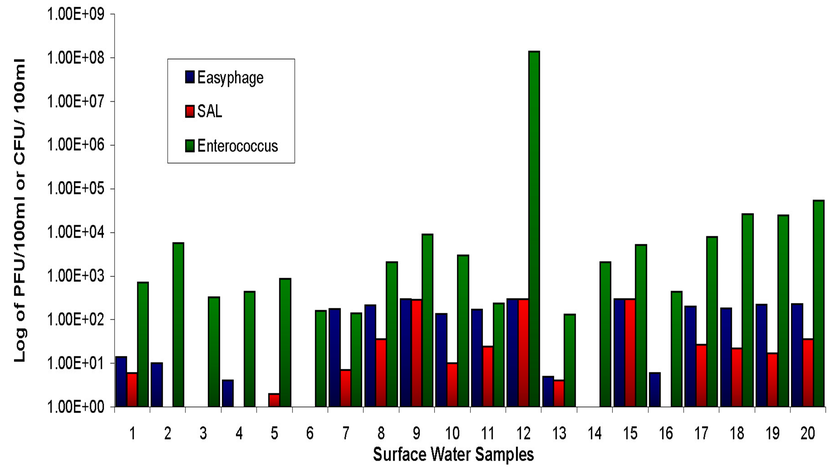
		SAL				Easyphage			
	No. of Samples	PFU/100ml	Mean	Recovery %	Mean	PFU/100ml	Mean	Recovery %	Mean
Spiked samples	25	2 to 20	5.7	3% to 25%	7%	46 to 78	55.5	58% to 98%	69%
Surface water	20	<1 to 284	53.8	–	–	<1 to ≥300	123.6	–	–
Sewage samples	2	600 to 1500	1050	–	–	2600 to 4000	3300	–	–
Blanks	15	<1	<1	–	–	<1	<1	–	–

Chart 1. Single Agar Layer and Easyphage F+ Coliphage Recovery Percentage in Samples spiked with 80 PFU/100mL



Results

Chart 2. Enterococcus and F+ Coliphage Recovery in Surface Waters



Discussion/Conclusions

- The pectin based Easyphage method was more effective in the recovery of F+ coliphage in sewage, spiked, and surface water samples than agar based SAL method.
- Sewage samples served as true positives with a mean recovery of 1050 PFU/100mL (600-1500) for SAL and 3300 PFU/100mL (2600-4000) for Easyphage. True negatives were observed with blanks and samples with no coliphage recovery in both methods (Table 1).
- In samples spiked with 80 PFU/100mL, SAL F+ coliphage recovery ranged from 2 to 20 PFU/100mL whereas Easyphage ranged from 46 to 78 PFU/100mL. The recovery percentage was 3% to 25% for SAL and 58% to 98% for Easyphage with a respective mean recovery of 5.7 (7%) and 55.5 (69%) (Chart 1).
- In surface water samples, Easyphage recovered up to 25 times more coliphage than SAL. In these samples, the recovery mean was 53.8 PFU/100mL for SAL and 123.6 PFU/100mL for Easyphage (Chart 2).
- A viral indicator is needed since bacterial indicators are inadequate in predicting viral pathogenic levels, as seen in this study. Enterococcus levels were high in all surface water samples even in samples with no coliphage recovery (<1) in both methods. Enterococcus levels ranged from 1.3 x 10^2 to 1.4 x 10^8 colony forming unit (CFU) per 100mL with a mean of 7.0 x 10^6 CFU/100mL (Chart 2).
- SAL is temperature dependent, labor intensive with the handling of molten media, and plaques can be difficult to read because media imperfections and bubbles can be mistaken for plaques.
- Easyphage is simple, less labor intensive, and plaques are easier to identify than SAL but is more expensive.
- There is an increasing interest in the use of FRNA coliphage in microbial source tracking techniques (MST) since human fecal waste can be distinguished from non-human by coliphage genotyping.
- Although coliphage has been accepted for monitoring water supplies and water treatment efficiency, further studies are required for coliphage to serve as an enteric viral indicator for water quality assessment.

Acknowledgements

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