

Enterophages, a group of phages infecting *Enterococcus faecalis*, and their potential as alternate indicators of human faecal contamination

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Characterization of *Enterococcus faecalis*-infecting phages (enterophages) as markers of human fecal pollution in recreational waters

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Human and chicken-specific *Enterococcus* phages: their prevalence and survival

Tasha M. Santiago-Rodríguez

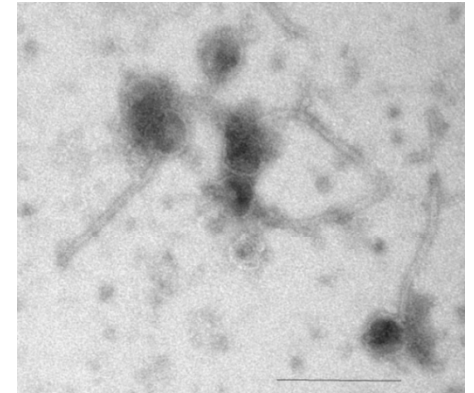
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University of Puerto Rico
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Introduction

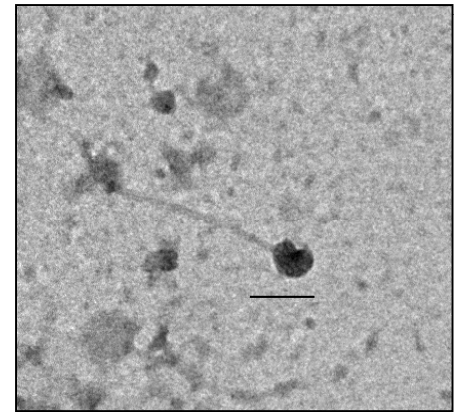
- Fecal contamination represents a health-related risk.
 - Possible introduction of pathogens.
 - Disadvantages of detecting pathogens.
- Microbial indicators
 - Disadvantages of currently used microbial indicators:
 - Thermotolerant coliforms → environmental microbiota
 - Enterococci → environmental microbiota
 - Coliphages → long inactivation
 - Source-specific ???
 - *Bacteroides fragilis* phages → limited to some geographical areas
 - Difficulties with detection technique (host is anaerobic)

Introduction

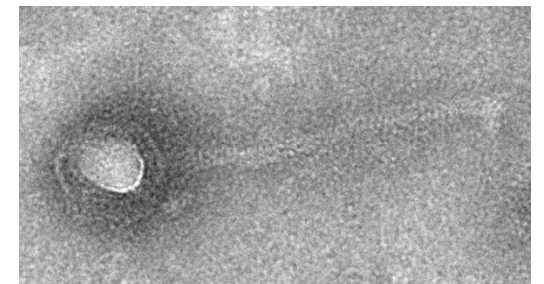
- New microbial indicators are needed:
 - Enteric viruses
 - Resistant to removal or inactivation methods.
 - EPA regulations of fast and reliable methods.
- *Enterococcus faecalis*-infecting phages (**enterophages**) as viral indicators of human fecal pollution.
- The methods for the detection of these phages in waters, sand and feces have been developed.
 - Specific inhibitory media.



Bar =200nm



Bar =20nm



Aims

- Further characterization of enterophages is still needed.
- It remains unknown if other *Enterococcus*-infecting phages exist.
- Therefore, the aims are to:
 - Determine the prevalence and survival of enterophages.
 - Rio Grande de Arecibo watershed.
 - Test different *Enterococcus* species as hosts for enterophages in sewage and feces.
 - Human
 - Chicken
 - Cattle

Rio Grande de Arecibo watershed



1. Lake Garza
2. Vaca river
3. Before WTP Adjuntas
4. After WTP Adjuntas
5. Rio Grande de Arecibo
6. Before WTP Utuado
7. After WTP Utuado
8. Mouth of Rio Grande de Arecibo
9. Criminales river
10. Caguana river

*WTP=Waste Treatment Plant

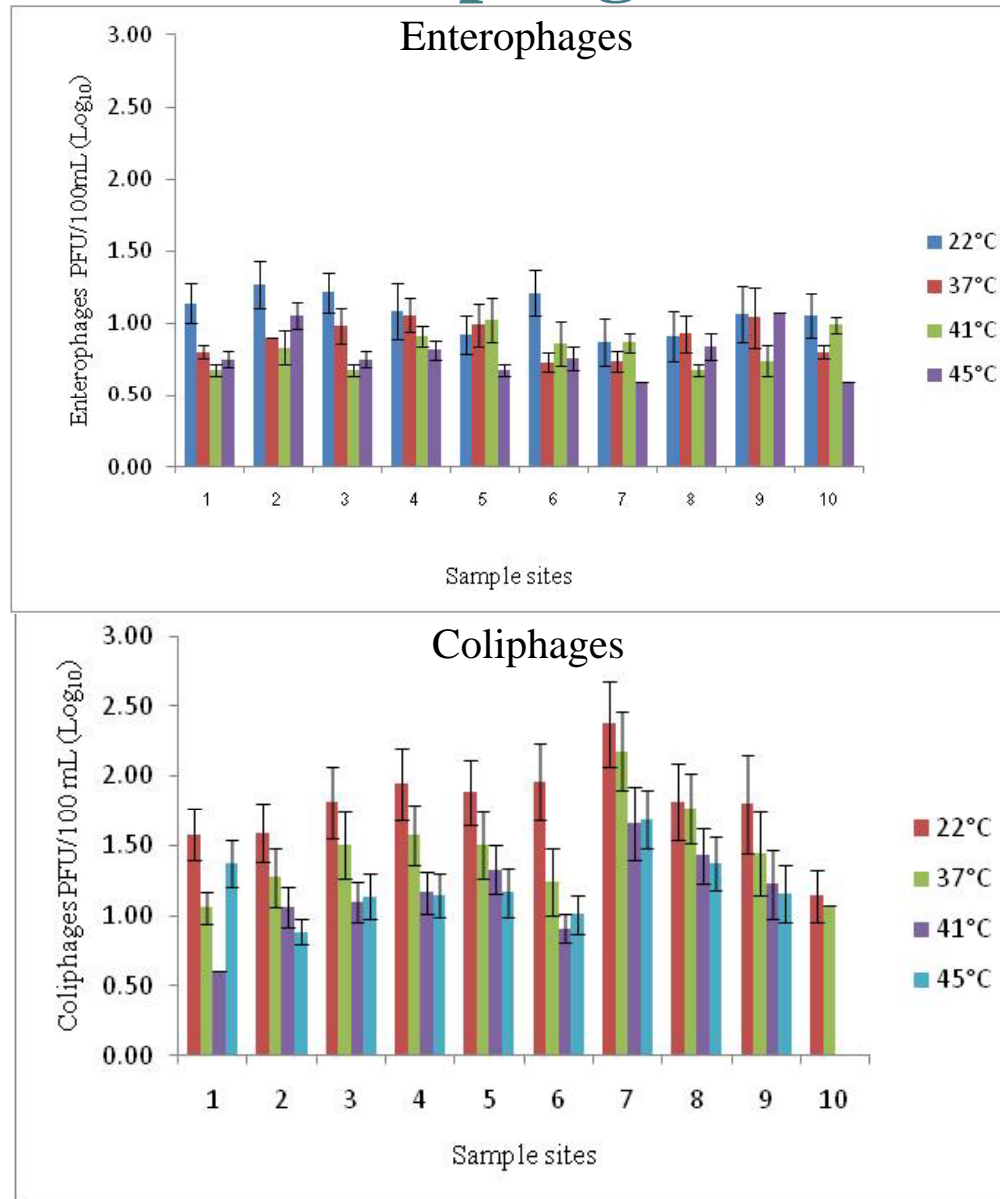
Methods

- Single layer method.
 - Prevalence of enterophages in the Rio Grande de Arecibo watershed.
 - Different incubation temperatures.
 - Comparison with other microbial indicators:
 - Coliphages
 - Enterococci
 - Thermotolerant coliforms
 - Survival of enterophages and coliphages in the Rio Grande de Arecibo watershed.
 - Lake Garza
 - After WTP at Utuado
 - Mouth of the Rio Grande de Arecibo
 - Addition of 10^4 enterophages and coliphages per 100mL from sewage.

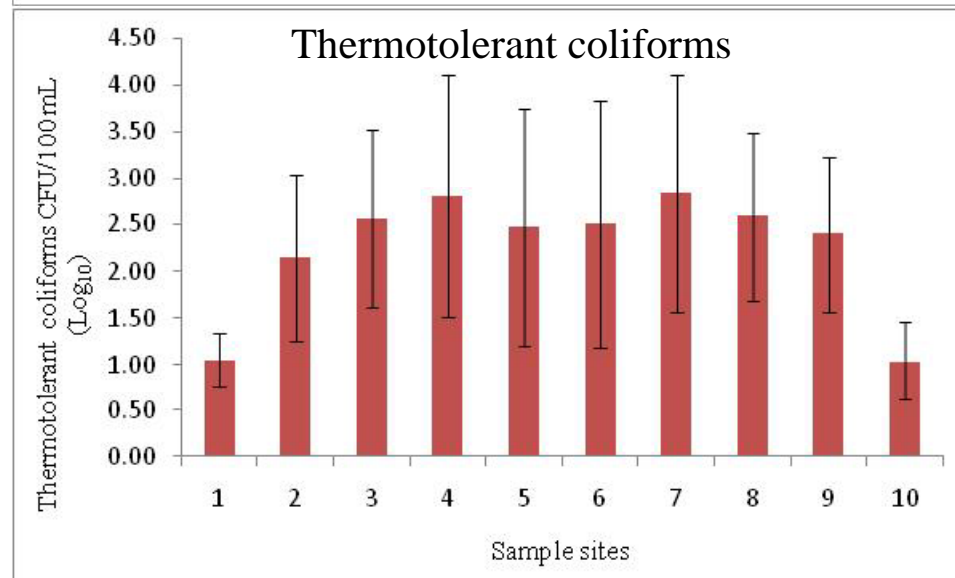
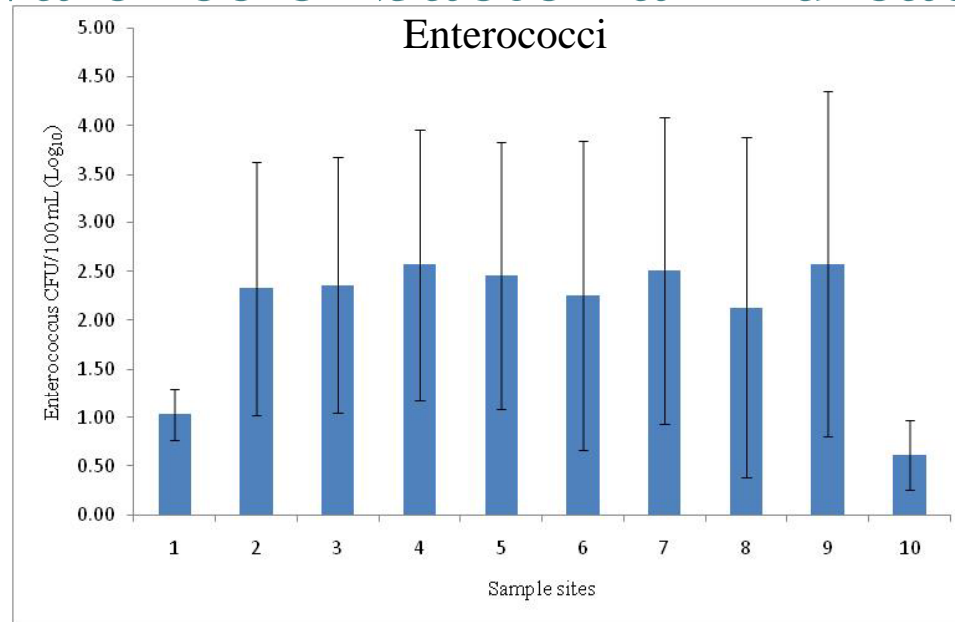
Methods

- Detection of phages using other *Enterococcus* species as hosts in human and animal feces and sewage.
 - E. faecium*
 - E. gallinarum*
 - E. hirae*
 - E. durans*
 - E. dispar*
 - E. casseliflavus*
 - E. pseudoavium*
 - E. faecalis*
- Detection of coliphages.
 - E. coli* C3000 (ATCC 15597)
- Incubation at different temperatures.

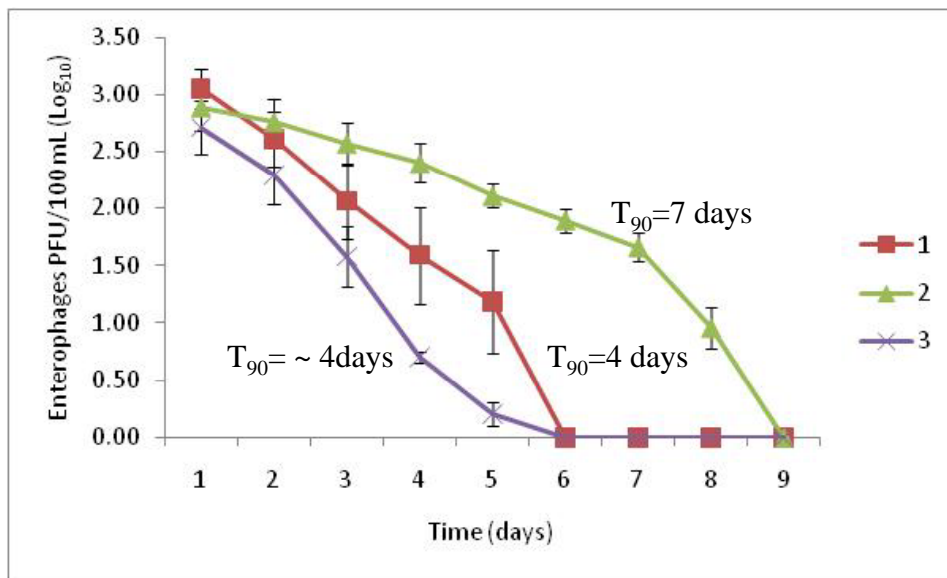
Prevalence of enterophages and coliphages



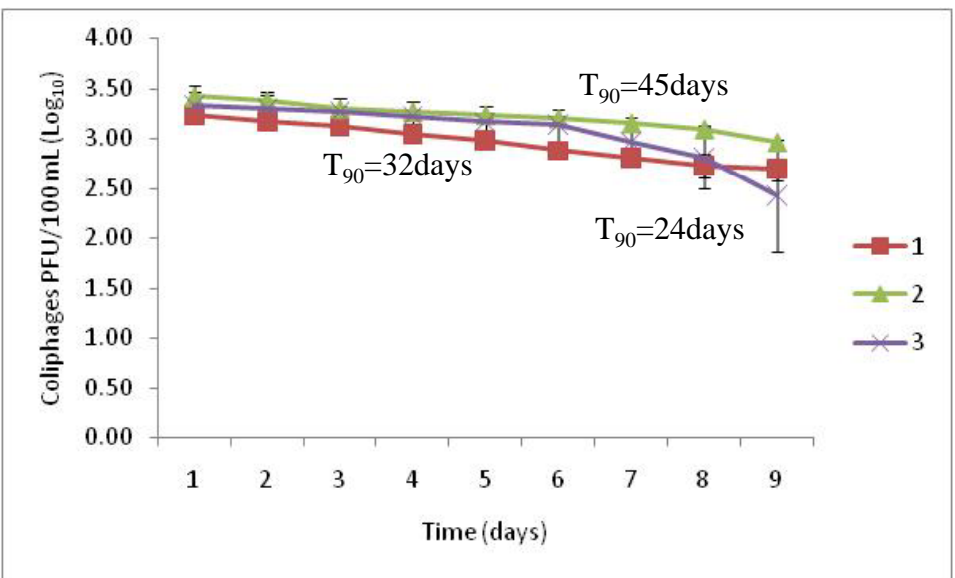
Prevalence of bacterial indicators



Survival of enterophages and coliphages



Enterophages



Coliphages

- 1-Lake Garza
- 2-After WTP at Utuado
- 3-Mouth of river

Coliphages and enterophages in feces and sewage

Source	Coliphages	<i>E. faecalis</i>
Human feces	7.2±4.0	4.0±2.8
Cattle feces	40.3±28.0	ND
Chicken feces	1.0±0.0	ND
Untreated sewage*		
22°C	-	22.0±20.0
37°C	1.8x10 ⁵ ±1.6x10 ⁵	65.0±72.15
41°C	-	5.0±7.1
Treated sewage*		
22°C	-	0
37°C	1.4x10 ³ ±1.3x10 ³	18±1.2
41°C	-	0

*One waste treatment plant

ND=Not detected

Other *Enterococcus* species as hosts in feces and sewage

Source	<i>E. faecium</i>	<i>E. casseliflavus</i>	<i>E. pseudoavium</i>	<i>E. gallinarum</i>	<i>E. durans</i>	<i>E. dispar</i>	<i>E. hirae</i>
Human feces	ND	ND	ND	ND	ND	ND	ND
Cattle feces	ND	ND	ND	ND	ND	ND	ND
Chicken feces	107.5±10.6	65.0±0.0	3.8±1.8	ND	ND	ND	ND
Untreated sewage*							
22°C	ND	44.0±62.2	31.0±43.8	ND	ND	ND	ND
37°C	200.0±141.2	26.0±18.3	ND	ND	ND	ND	ND
41°C	56.0±79.2	ND	ND	ND	ND	ND	ND
Treated sewage*							
22°C	ND	8.0±5.7	1.0±1.4	ND	ND	ND	ND
37°C	0	2.0±2.8	ND	ND	ND	ND	ND
41°C	0	ND	ND	ND	ND	ND	ND

*One waste treatment plant

ND=Not detected

Conclusions

- 1. Enterophages have the potential of microbial indicators of human fecal pollution.**
 - Their prevalence suggest:
 - Recent fecal contamination.
 - Their inactivation is similar to that of enteric viruses in fresh waters.
 - Present in human feces and absent in animal feces.



2. The characterization of other *Enterococcus*-infecting phages is still needed.

- Host specificity
- Source specificity
 - Temperature??
- Morphology
- Nucleic acids
- Potential as microbial indicators of other fecal sources ??

Thanks to Enterophages group



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