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Julia Chen
Illinois Wesleyan University

Ria Patel
Illinois Wesleyan University

Julie Xu
Illinois Wesleyan University

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Newly Discovered Phage *Xuper*: Single Ready to Mingle with *Rhodobacter capsulatus*

Julia Chen, Ria Patel, Julie Xu, Richard Alvey

Illinois Wesleyan University, Biology Department, Bloomington, Illinois 61701

Introduction:

Bacteriophages, viruses that infect and replicate in bacteria, are the most abundant biological entities in the biosphere. Despite their prevalence, the evolutionary relationships between these viruses as well as the functions of many phage genes remain unknown. The goal of this study was to characterize and genomically analyze a new phage in order to learn more about its unique features, as well as explore the vastly diverse evolutionary relationships of these viruses. In our investigation, we isolated *Xuper* from an environmental water sample using the bacterial host *Rhodobacter capsulatus*. A series of experiments were performed, including host range testing, lysogen/immunity testing, transmission electron microscopy, and DNA sequencing. Based on the distinctive morphology and genome, we concluded that *Xuper* is a singleton.

Diversity of Phage Morphologies Isolated:

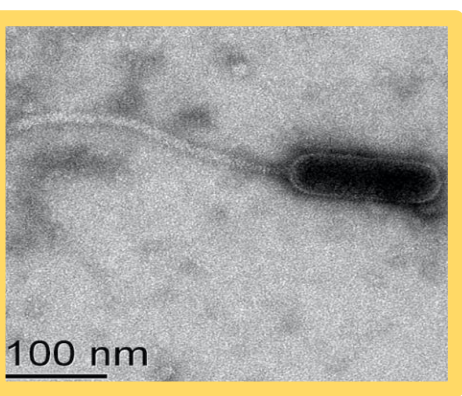


Figure 1. Xuper (Rc)

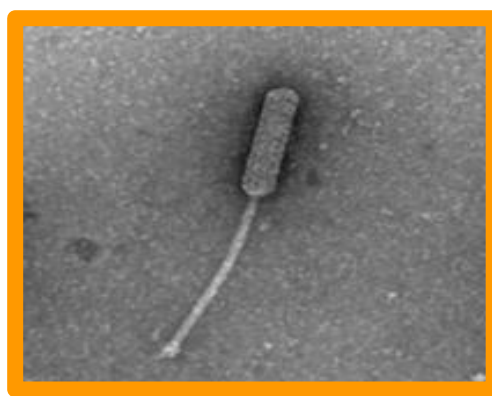


Figure 2. Corndog (Ms)

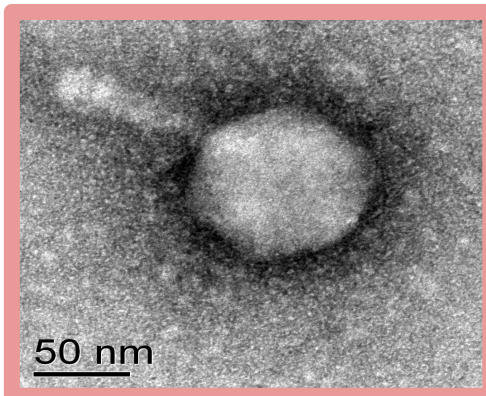


Figure 3. Lionheart (Ms)

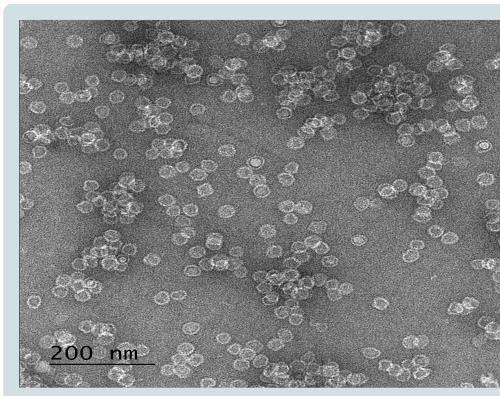


Figure 4. SchuylerLagoon (Rc)

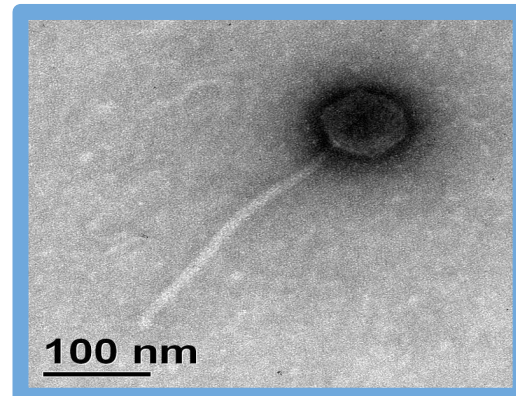


Figure 5. Tiptonus (Rc)

Table 1: Comparison of Morphology and Genome Length

Phage Name:	Capsid Diameter (nm)	Tail Length (nm)	Genome Length (kb)	Shape of Capsid
Xuper	50 x 147	304	148	Prolate
Corndog	42 x 169	268	70	Prolate
Lionheart	98	82	155 (predicted)	Icosahedral
SchuylerLagoon	31	0	5 (predicted)	Icosahedral
Tiptonus	84	291	96	Icosahedral

Collection Site:

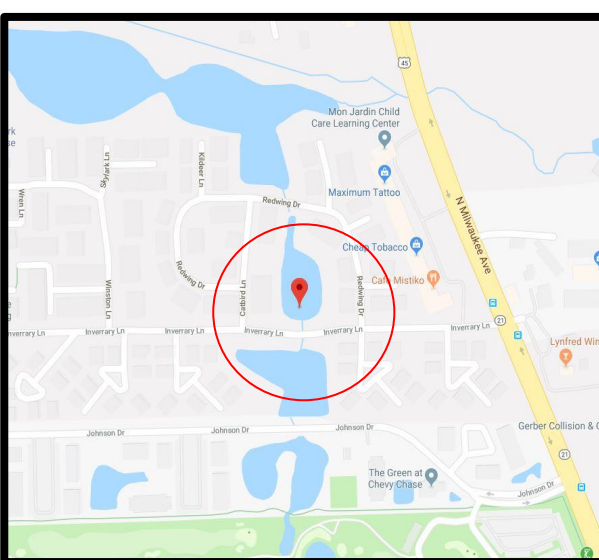


Figure 6. Collection Site of Xuper



The sample was collected at a neighborhood pond in Deerfield Illinois (42.16091, -87.92213) at a temperature of 19°C. At time of collection, there were many aquatic organisms present.

Plaque Morphology:

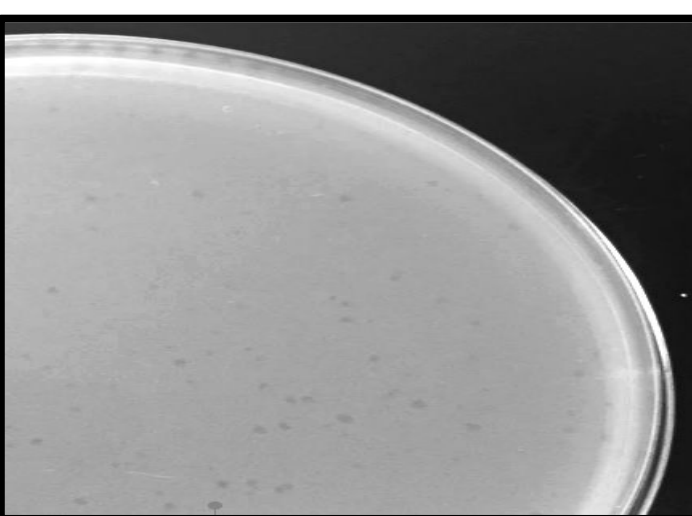


Figure 7. Xuper Plaques

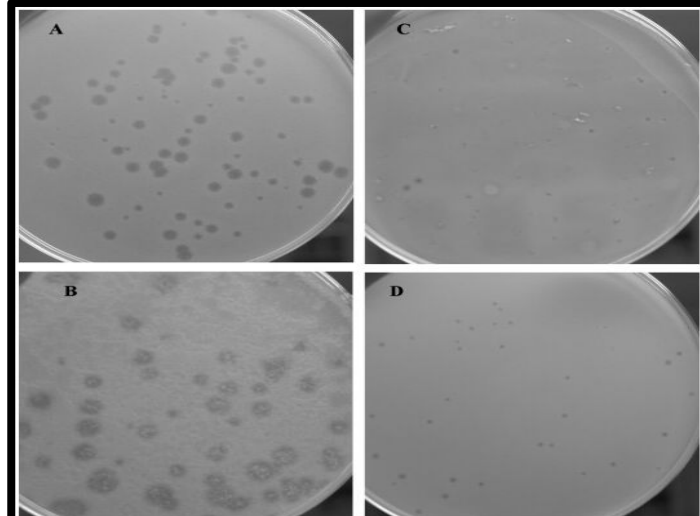
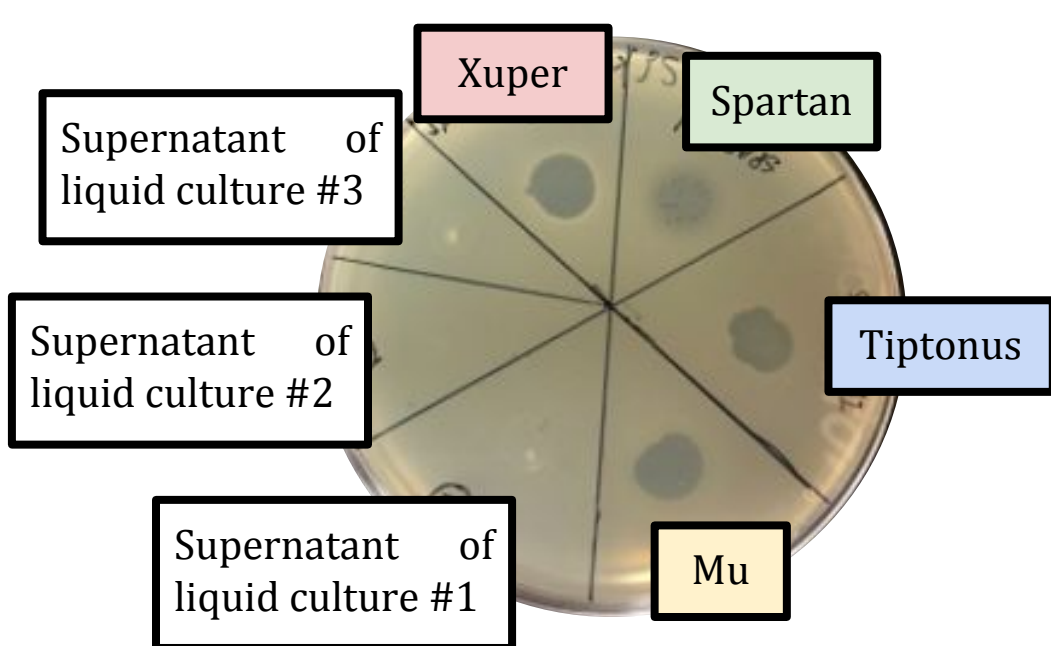


Figure 8. Variations of Plaques

By studying and distinguishing the morphology of plaques, we can determine whether a phage is virulent or temperate. Although there are exceptions, virulent phages tend to form clear plaques and temperate phages tend to form cloudy/turbid plaques. The plaque morphology of Xuper is relatively small (0.5mm in diameter) and clear, suggesting it is a virulent phage.

Lysogen Testing:

Figure 10. Control Plate



Three rounds of lysogen testing was performed on Xuper in an attempt to isolate an infection-resistant bacterial lysogen. Figure 10 serves as the positive control using *R. capsulatus* to ensure that the phages of interest were able to successfully infect the host. The positive control was also used to indicate if the possible lysogen leaks phage. Each of the isolated bacterial colonies were then challenged by Xuper in order to prove whether or not a lysogen formed; three other phages were also used to reveal possible relatedness (if a lysogen formed). In all three rounds of lysogen testing, the same results were obtained: Xuper was unable to form stable lysogens.

Figure 11. Results

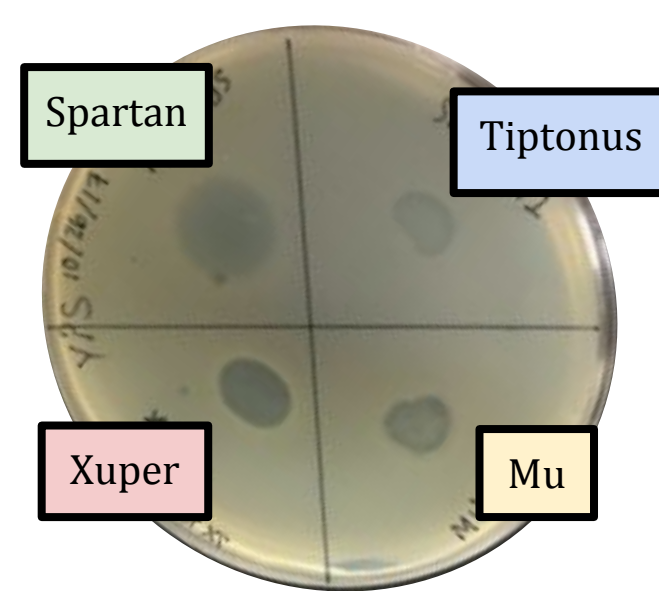


Table 2: Host Range Testing Results

	YW1	YW2	B6	B10	St.Louis	37B4	Iona
Xuper	X	X	X	X	X		
Pacific	X	X					
Waterboi	X	X			X		
RcBaka	X		X	X	X		

Based on the host range results, Xuper has a wide host range infecting 5 different strains of *R. capsulatus* compared to the three other phages that host range testing was performed on. Xuper is able to infect YW1, YW2, B6, B10, and St. Louis, but was unable to infect strains 37B4 and Iona.

Table 3: Genome Comparisons

	Xuper	Tiptonus	Corndog
Genome Length (bp)	147,673	95,520	69,777
Number of Genes	224	148	99
Number of tRNAs	36	0	0
%GC	61.9%	58.0%	65.4%

Xuper and Tiptonus are singletons. Even though Tiptonus is Xuper's closest relative there are many differences that exist between these two phages.

Genome Analysis

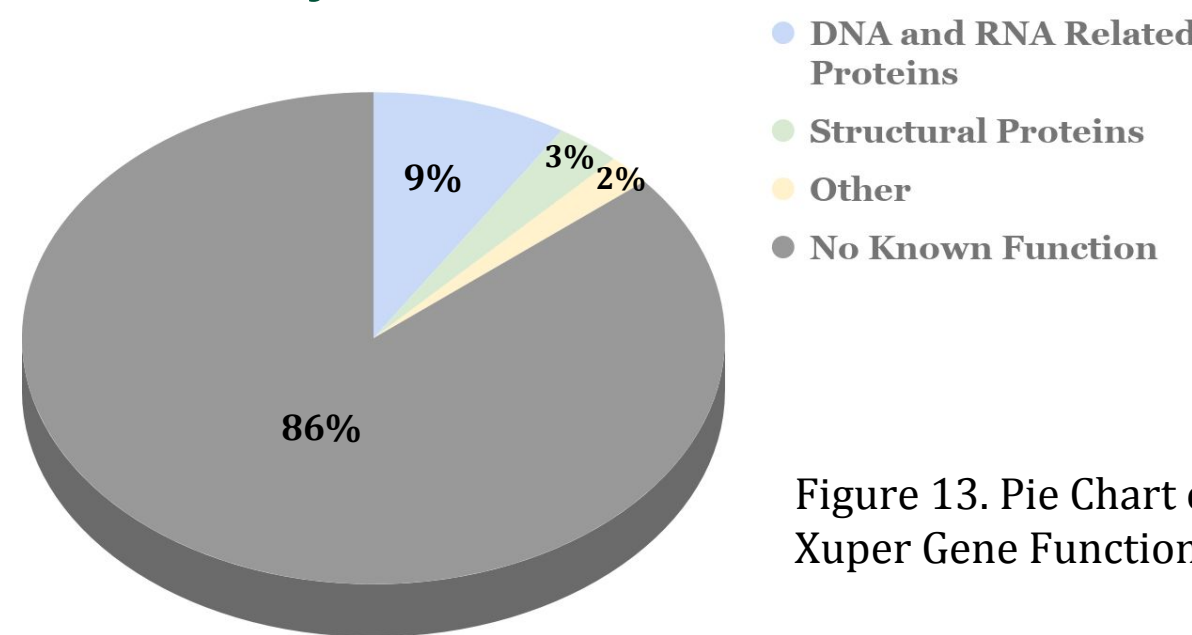


Figure 13. Pie Chart of Xuper Gene Functions

The majority of Xuper's genes had no known function when compared to other phage genomes, a common characteristic of singletons. Out of the genes that were assigned functions, a few were shared with Tiptonus. Interestingly, no genes appeared to be shared with any members of the RcB cluster or RcapMu.

Conclusion:

Xuper is a singleton with many unique features. It is the first isolated *R. capsulatus* phage with an elongated prolate capsid and forms exceptionally small plaques. It was not observed to form a stable lysogen. However, splitstree analysis demonstrated that Tiptonus is Xuper's closest relative. Additionally, Xuper is the only discovered *R. capsulatus* phage to have its own tRNAs and has the largest genome of any *R. capsulatus* phage discovered thus far.

Acknowledgements:

Special thanks to Howard Hughes Medical Institute for providing us with this opportunity and Dr. Alvey for guiding us in our phage hunting journey.

Phamerator Map:

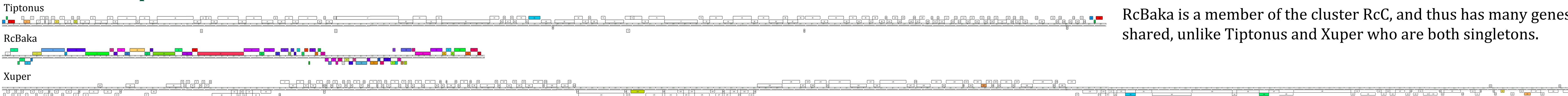


Figure 9. Phamerator map comparing Xuper, RcBaka, and Tiptonus. The presence of color on the map indicates genes shared with other phages.

RcBaka is a member of the cluster RcC, and thus has many genes that are shared, unlike Tiptonus and Xuper who are both singletons.