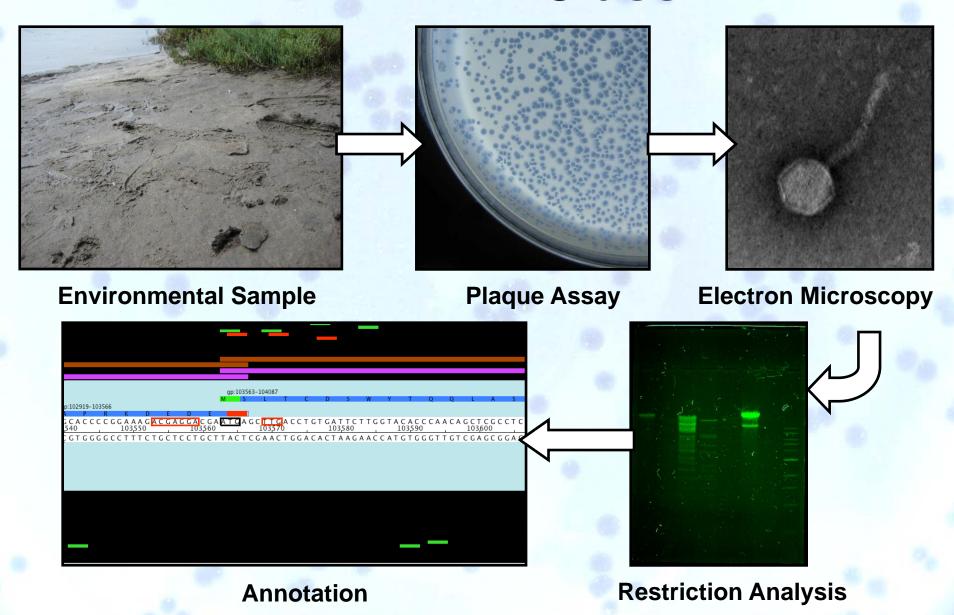
The Isolation and Characterization of Novel Mycobacteriophages in San Diego

Hannah Wang
University of California, San Diego
June 21, 2009

BIMM 171 Class



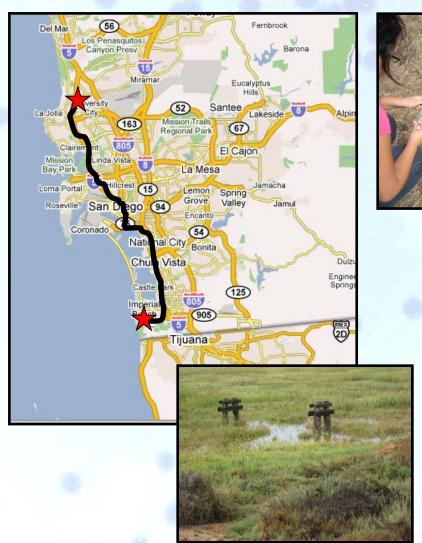
Isolation Overview

- 491 direct plating
- 33 enrichments
- 21 samples yielded plaques from direct plating (4%)
- •16 samples yielded plaques from enrichment (48%)
- 19 novel phage isolated

Isolation Overview

#	PHAGE	OWNER(S)	#	PHAGE	OWNER(S)
1.	Auxin	Andrew A.S. Ang	12.	Fun Box	Jason Ho & Theresa Wong
2.	Үо-уо	Kayla Busby & Simmi Deo	13.	Snuggles	Diana T. Li
3.	Grass Blade	Sahrish Ekram	14.	ET08	Tina Lu
4.	Sunny Delight (previously isolated as Orange Drop)	Crystal Estrada	15.	Helianthus	Emilie Nguyen
5.	Sun God Bones	Shannon Fox	16.	Anina	
6.	Solar 36	Ethan Fram & Philip Wu	17.	Diego	Katrina Nguyen & Hannah Wang
7.	Lemon Drop (previously isolated)	Bridget Guiza	18.	Dora	s. Harman Traing
8.	Lowlife (previously isolated)	Auroni Gupta & Yun Jeong Yang	19.	Kanye South	J. Bradley Segal
9.	RingO	Leila Haghighat	20.	Tree Hollow	Tin-Yun Tang
10.	Freckles		21.	Tin	Jon Tsay
11.	Wilmo	Kevin He	22.	Cherry	Danielle Wang

Phage Phinding Trial 1



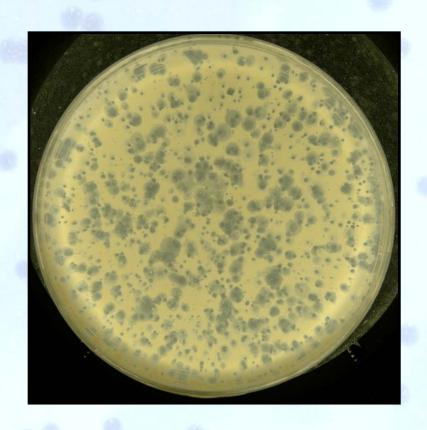






Phage Phinding Trial XX4





- All but one positive site was from compost
- No compost samples were plaque negative

Plaque Purification



Plaque Morphologies



Clear Lytic

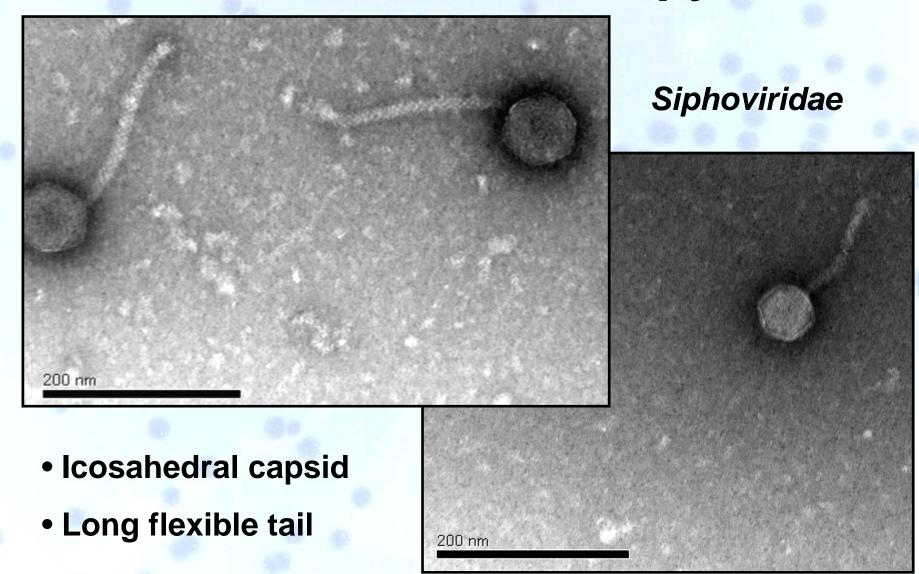


Turbid Lysogenic

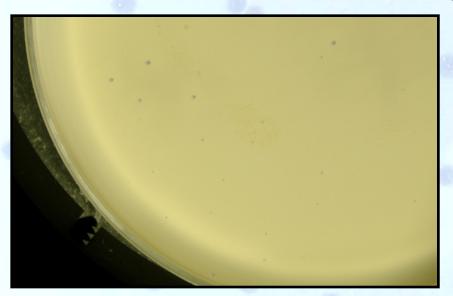


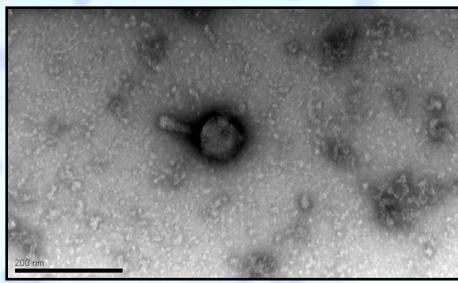
Bulls-Eye ???

Electron Microscopy



ET08: Myoviridae





Plaque Morphology

- small
- dot-like
- clear

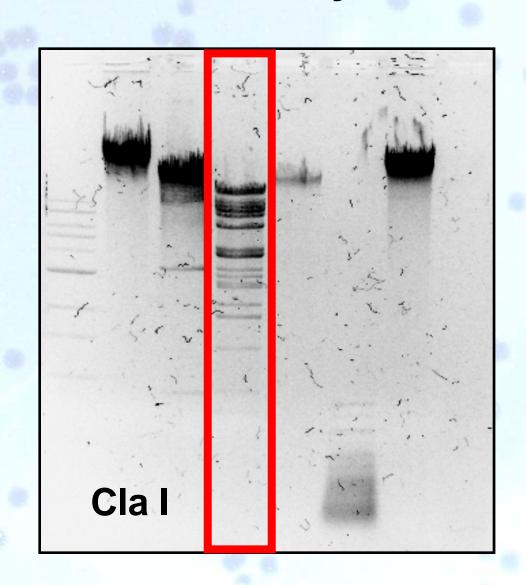
Electron Microscopy

- contractile tail
- capsid diameter ~80 nm
- tail length ~80 nm

DNA Restriction Analysis



~155,445 base pairs

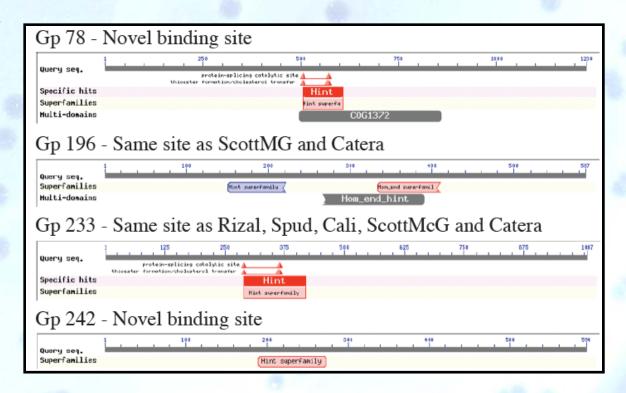


Annotation of ET08

- Glimmer and Genemark in Apollo identified ORFs
- Manually evaluated Shine Delgarno Sites and BLASTp matches
- Member of Cluster C1 contractile tailed mycobacteriophage family
- Phamerator showed 1 ET08 gene product to be novel phamily
- tBLASTn showed that this gene was not actually novel genetic information
- Extensive genetic similarity to other Cluster C1 phage

Novel Elements of ET08 Genome

- 4 inteins
 - 2 with conserved binding sites
 - 2 with novel binding sites
- 33 putative tRNAs
- 1 tmRNA



Conclusion

1. Isolation of environmental samples and plaque purification

- 2. Characterization through
 - Plaque assays
 - Electron microscopy
 - DNA restriction analysis

3. Annotation and identification of novel inteins and tRNA



HHMI HOWARD HUGHES MEDICAL INSTITUTE



Marcella Erb, Dr. Joseph Pogliano, Dr. Kit Pogliano, Dr. Madeline Butler, Dr. Lorlina Almazan, Howard Hughes Medical Institute, UCSD Department of Biology, VA San Diego Medical Center Electron Microscopy Facilities, Andrew A.S. Ang, Kayla Busby, Simmi Deo, Sahrish Ekram, Crystal Estrada, Shannon Fox, Ethan Fram, Bridget Guiza, Auroni Gupta, Leila Haghighat, Kevin He, Jason Ho, Diana T. Li, Tina Lu, Emilie Nguyen, Katrina Nguyen, J. Bradley Segal, Tin-Yun Tang, Jonathan Tsay, Danielle Wang, Theresa Wong, Phillip Wu, Yun jeong Yang