NEW RHODOCOCCUS AND ARTHROBACTER PHAGES: ANNOTATION, LYSOGENS, REGULATORY ELEMENTS AND UNUSUAL SEQUENCE COVERAGE BEHAVIOR WITHIN CLUSTER AT.

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BACKGROUND & SUMMARY

Bacteriophages are the most abundant biological entities on earth, with important ecological impact as well as biomedical applications. To improve our understanding of their biology and evolution, we searched for new phages that infect Actinobacterial hosts *Rhodococcus erythropolis* or *Arthrobacter sp*. Arthrobacter has potential applications in bioremediation, whereas Rhodococcus can compromise wastewater treatment plants but has potential applications as a whole-cell catalyst.

• We discovered 3 Rhodococcus phages and 9 Arthrobacter phages, which we sequenced on the Ion Torrent PGM platform (Panel A). One Rhodococcus phage (UhSalsa) and one Arthrobacter phage (Swenson) were also sequenced on the Illumina Platform and assembled at University of Pittsburgh. These two genomes were completely annotated (Panels B and C). The remainder were assembled using SPades and partially annotated pending sequence finishing (Panel A).

• We investigated Rhodococcus phage UhSalsa in more detail, confirming experimentally that it is a temperate phage (Panel D) and identifying candidate transcriptional elements associated with its integrase, excisionase and repressor genes (Panels E and F).

• Although the other two known members of cluster AT exhibited extremely biased read coverage upon Illumina sequencing (>95% reverse strand: phagesdb.org), our closely related phage BeatusComendenti (Panel A) did not exhibit strand coverage bias in IonTorrent sequencing (Panel G). However, sequencing the same DNA sample on the Illumina platform (Daniel Russell, U. Pittsburgh) resulted in the same bias as for KitKat and KellEzio (not shown). This suggests that a feature or modification of the cluster AT genomes results in a platform-specific sequencing bias.

A) Phages Isolated in this Study

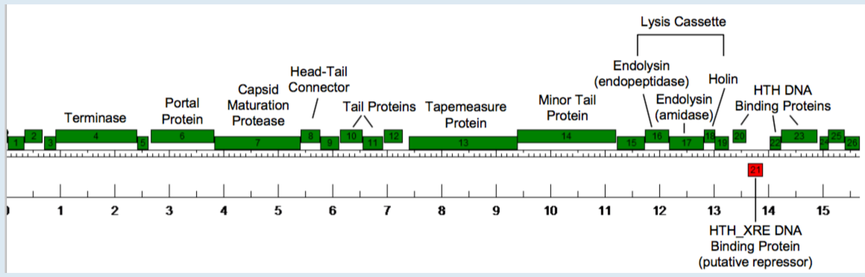
Phage Name	Genome Size (bp)	Most similar phage (NCBI BLAST)	% Identity	Cluster	Morphotype
Amici	46510	Rhodalysa	98	CA	Siphoviridae
IngentiaLocuti	46438	Rhodalysa	99	CA	Siphoviridae
UhSalsa	46539	Rhodalysa	99	CA	Siphoviridae
BingBing	43415	Wayne	98	AK	Siphoviridae
Friendshipgu	42870	Vulture	97	AK	Siphoviridae
Swenson	15680	Sandman	97	AN	Siphoviridae
Satisfachen	51297	BarretLemon	98	AO	Myoviridae
ArhuroReed	69991	PrincessTrina	99	AR	Siphoviridae
Hongidius	70257	PrincessTrina	99	AR	Siphoviridae
Beatus Comendenti	58762	KitKat *	99	AT	Siphoviridae
BuchananBCBio	58919	Gordon	92	AU	Siphoviridae
ComptonP	58917	Gordon	92	AU	Siphoviridae

* Kitkat: 99% identity over 98% coverage; KellEzio: 98% identity over 95% coverage

RESULTS AND DISCUSSION

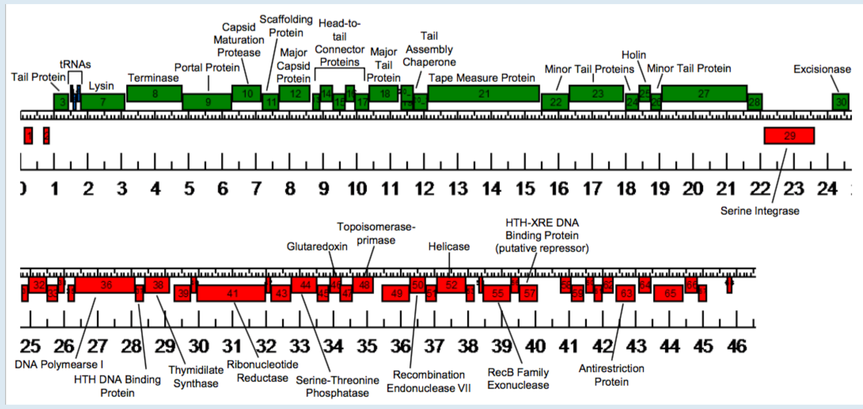
B) Arthrobacter Phage Swenson: Genome Annotation

Glimmer, GeneMark and human annotation in DNAMAster
Functions from NCBI BLAST, CDD, HHPred

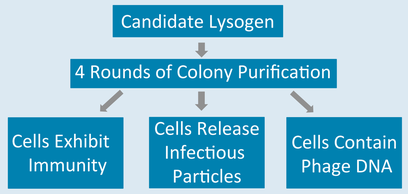


C) Rhodococcus Phage UhSalsa: Genome Annotation

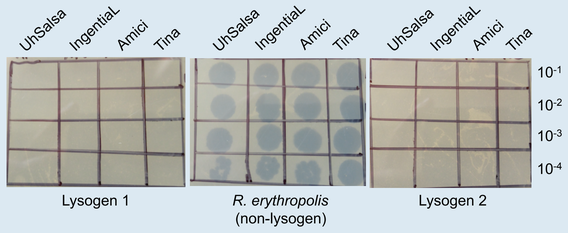
Glimmer, GeneMark and human annotation in DNAMAster
Functions from NCBI BLAST, CDD, HHPred



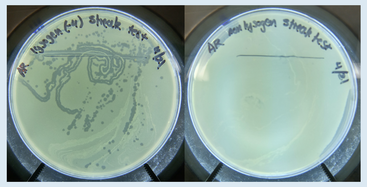
D) Rhodoccocus phage UhSalsa is temperate



(1) UhSalsa lysogens are immune to UhSalsa and related CA phages: Spot tests

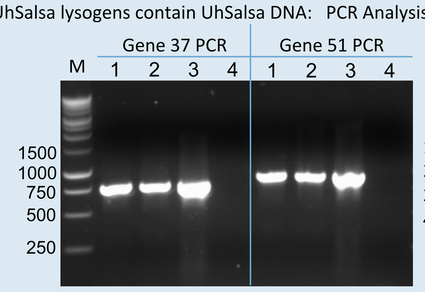


(2) UhSalsa lysogens release infectious particles: *R. Erythropolis* lawns poured over bacterial streaks

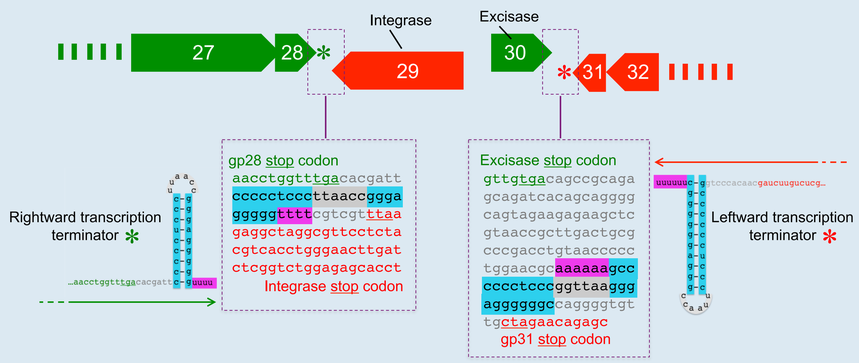


Lysogen 1 streak *R. erythropolis* streak

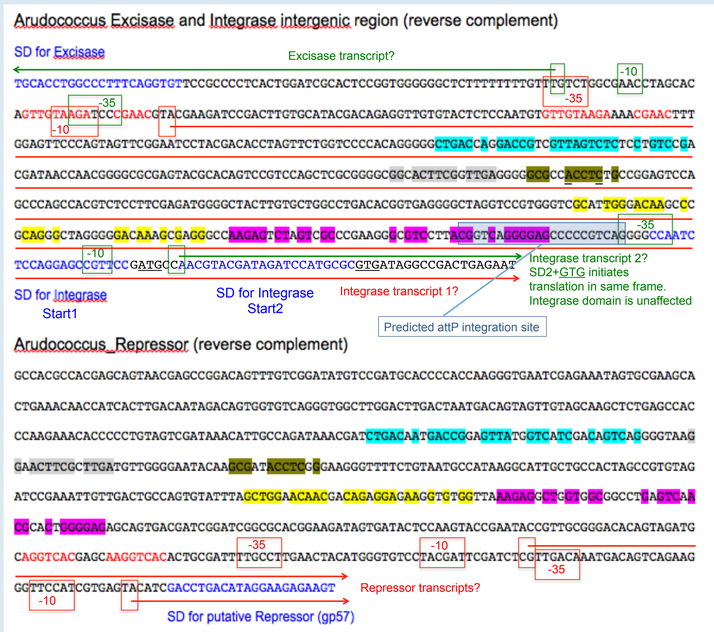
(3) UhSalsa lysogens contain UhSalsa DNA: PCR Analysis

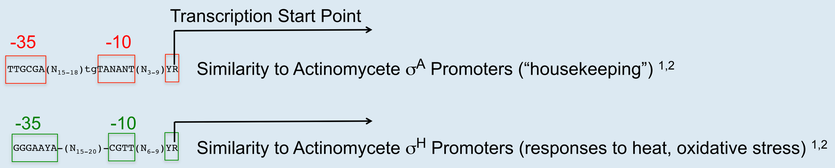


E) Terminators for Rightward and Leftward Transcription Flank the Integrase and Excisase Genes at the Center of UhSalsa Genome



F) Candidate Promoters and Possible Regulatory Elements Near Lysogeny Genes of UhSalsa





1. Engels et al (2004) *Mol Microbiol* 52:285-302
2. Patek M. and Nesvera J. (2011) *J. Biotechnol* 154:101-113

• Is excision of UhSalsa prophage induced by heat or oxidative stress?

• Is integration vs excision controlled by promoter competition and switching?

No stress: σ^A promoters active → Repressor + Integrase1 → Integration

Stress: σ^H promoters active → Excisase + Integrase2 → Excision

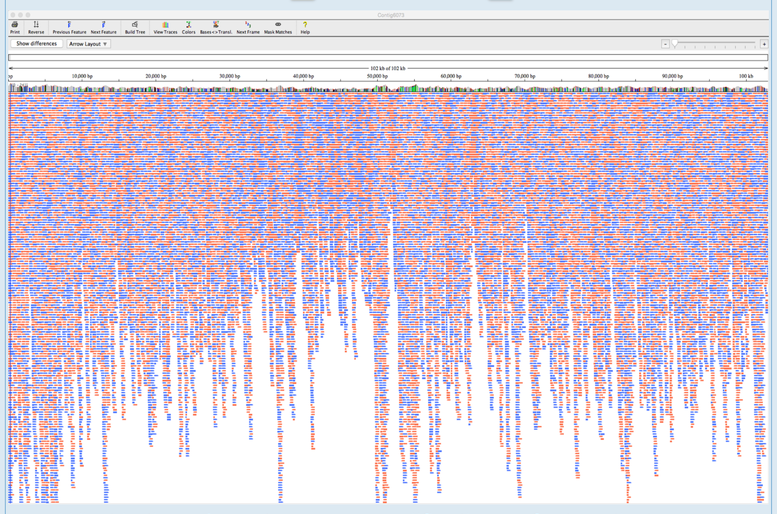
Integration separates the Integrase ORF from the σ^A promoter but not σ^H, so Integrase can be expressed for excision under stress conditions

Sequences shared upstream of the Integrase and Repressor ORFs. Conserved among wide range of Rhodococcus phages.

• Possible regulatory elements for control of lysogeny?

G) Arthrobacter Phage BeatusComendenti: Ion Torrent read distribution does not exhibit strand bias, although Illumina reads are strongly biased to bottom strand as for KitKat and KellEzio

Analysis of IonTorrent Reads in CodonCode Aligner. Average Read length 200 bp
Average Coverage: 248.6 Forward reads Reverse reads



What is the relevant genome modification or feature that causes this platform-specific behavior of AT phage genomes?

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• Daniel Russell (U.Pittsburgh): Illumina sequencing of BeatusComendenti