Phaging on the River: Further Adventures in South Louisiana Phage Biology

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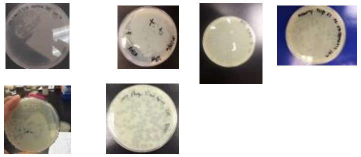
Abstract #:84:

Phages were isolated from a variety of locales in and around the Greater New Orleans, Louisiana area using standard microbiological techniques. When allowed to form plaques in a soft-agar overlay culture with *M. smegmatis* mc^{2}155 as host, phages displayed a variety of plaque sizes and morphologies. Titers of lysates varied from roughly 10^8 to 10^{14} plaque-forming units per milliliter. One phage, LilDestine, was isolated from the vicinity of Norco, Louisiana near a large refinery, and this phage was selected for sequencing. The LilDestine genome is somewhat more than 75,000bp in length, with cohesive ends showing a ten base pair overlap. BLASTn analysis reveals considerable nucleotide homology with the genomes of other known mycobacteriophages, including Wilder, Winky, Breezona, Faith1, Crossroads, Loadrie and Nicholasp3. These homologies support assignment of LilDestine to the L cluster and specifically the L2 subcluster. DNA Master auto-annotation employing Glimmer and GeneMark called about 140 total features. Analysis with Aragorn via the World Wide Web, external to the DNA Master environment, called 12 tRNA-encoding genes. These tRNA calls are located in a single cluster around 62-63kbp from the left end, except for two that are a bit further down, around 65kbp. All code for standard amino acids, with no tmRNAs called. A scan employing tRNAscanSE returns results that are in substantial agreement with the Aragorn data. In the case of protein encoding genes, in those cases where there is sufficient support from BLASTp and other sources (e.g. the Conserved Domain Database [via phamerator]) we suggest a possible function.



L to R from upper left, LilDestine, Ferociterium, Sphynx18, MoCo96, Delilah23, Freter5. L agar with 7H9 soft agar overlay.

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Results and Discussion

A total of sixteen phages were archived. At lower left are plaque photos from a selection of our isolates. Plaques varied over a wide range of sizes and appearances. LilDestine appears (via BLASTn [1]) to be very similar to such other L2 subcluster [3] phages as Wilder, Winky, Crossroads and Breezona. In LilDestine, DNA Master called about 140 total features, while external Aragorn [4] called 12 tRNA genes, mainly located in a cluster from about 62-63 kbp from the left. The Aragorn data are in substantial agreement with those provided by tRNAScanSE (5). Very similar, though not identical, clusters of tRNA genes are found in Breezona, Crossroads, Faith1 and Winky (data not shown), about 500-1000bp further downstream than in the case of LilDestine's cluster. BLASTp analysis suggests possible functions for a number of putative gene products. In the majority of cases (data not shown) the phamerator database (2) shows relevant CDD functional domains. In the table at right we show a selection of these suggestions in comparison to those proposed in the published annotations of several presumably related genomes. In addition to showing great structural similarity between the genome of LilDestine and these published genomes, these data suggest a fair degree of regional differentiation, as is very commonly seen in bacteriophage genomes. One side of the genome appears to be heavily involved in what could be called structural or "late" functions (e.g. nucleocapsid assembly and host cell lysis), while the other side is not only more heterogeneous but also seems to be the province of genes encoding catalytic and/or regulatory functions. The data also suggest that it is possible that this virus is capable of pursuing a temperate life cycle. We have not ourselves observed this: the plaque is normally clear, and while there is strong BLAST support for the presence of an integrase, other functions required for this way of life such as repressors and the like, while suggested perhaps, are less well supported.

CONCLUSIONS AND POSSIBLE FUTURE DIRECTIONS

1. LilDestine has much in common genetically with other members of its cluster, and it seems likely that it is capable of pursuing a temperate lifecycle.
2. The next logical step in exploring the biology of this virus is to determine if lysogeny is in fact possible in this system.
3. We would also like to do a bioinformatic search for promoters and transcriptional terminators and perhaps begin to identify possible operons.

Selected possible functions, LilDestine and four relatives:
Numbers show 5-prime coordinates of genes

POSSIBLE FUNCTION	LILDESTINE	BREEZONA	CROSSROADS	FAITH1	WINKY
Terminase large subunit	c. 1069bp	1069bp	1069 bp	1069bp	1069bp
Portal protein	2638	2638	2638	2638	2638
Major capsid protein	6070	6070	6070	6070	6070
Major tail protein	9002	9002	9002	9002	9002
Tape-measure	10,955	10,856	10,856	10,856	10,856
Minor tail protein	16,073	16,074	16,074	16,074	16,074
LysB	25,302	26,303	26,303	26,303	26,303
Integrase	31,654	30,751	31,657	31,656	31,656
Helicase	47,613	47,580	47,655	47,580	47,580
WhiB family transcr. Reg.	53,888	53,918	54,053	53,918	53,918
RNA ligase	55,893	56,203	56,278	56,203	56,203
HNH endo-nuclease	59,128	59,185	59,527	59,452	59,815
AAA ATPase	71,926	Not predicted	Not predicted	Not predicted	Not predicted

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