GENOMIC AND MORPHOLOGIC CHARACTERIZATION OF THE *MYCOBACTERIUM SMEGMATIS* BACTERIOPHAGE EAGLE

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BACKGROUND

- Course taught with 3 sections
- Started with enrichment protocol
- All students isolated new bacteriophages from environment

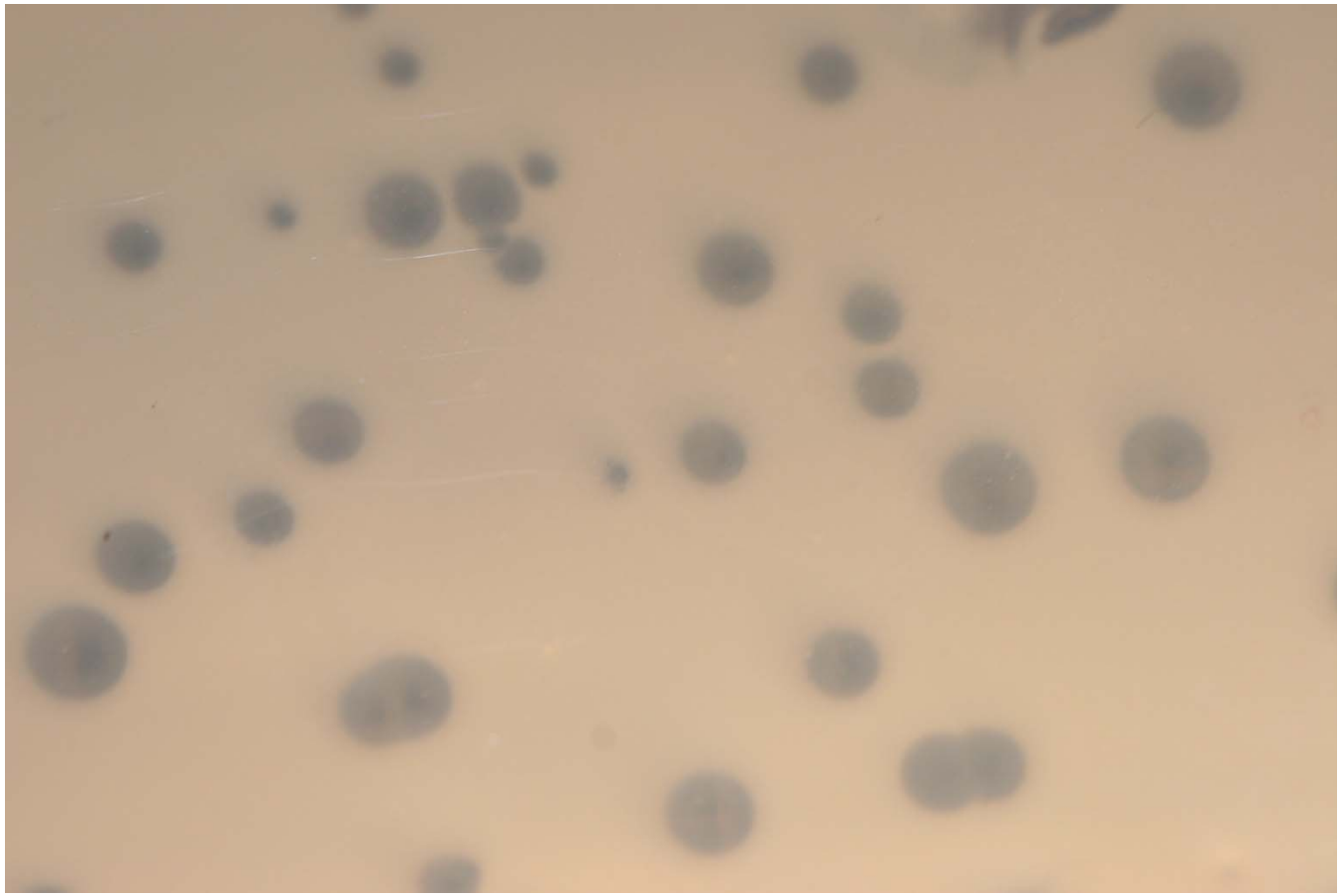
ENRICHMENT

- Soil samples were collected from the UMW campus
- 1 sample from each group of 3 students used for enrichment
- Enrichment protocol of Erin Sanders-Lorenz (UCLA) followed

PHAGE ISOLATION

- Plaques were picked after 24 hours incubation at 37ºC, using micropipette tip
- Phages resuspended in 100 μL phage buffer
- 10-fold dilutions performed and plated
- Plaques picked after 24 hr incubation, resuspended and plated

EAGLE PLAQUES



PHAGE ISOLATION (CONT'D)

- All plaques picked through at least three iterations to ensure purity
- Final plates were counted to determine pfu/mL of phage preparation
- 10 plates prepared for web lysis

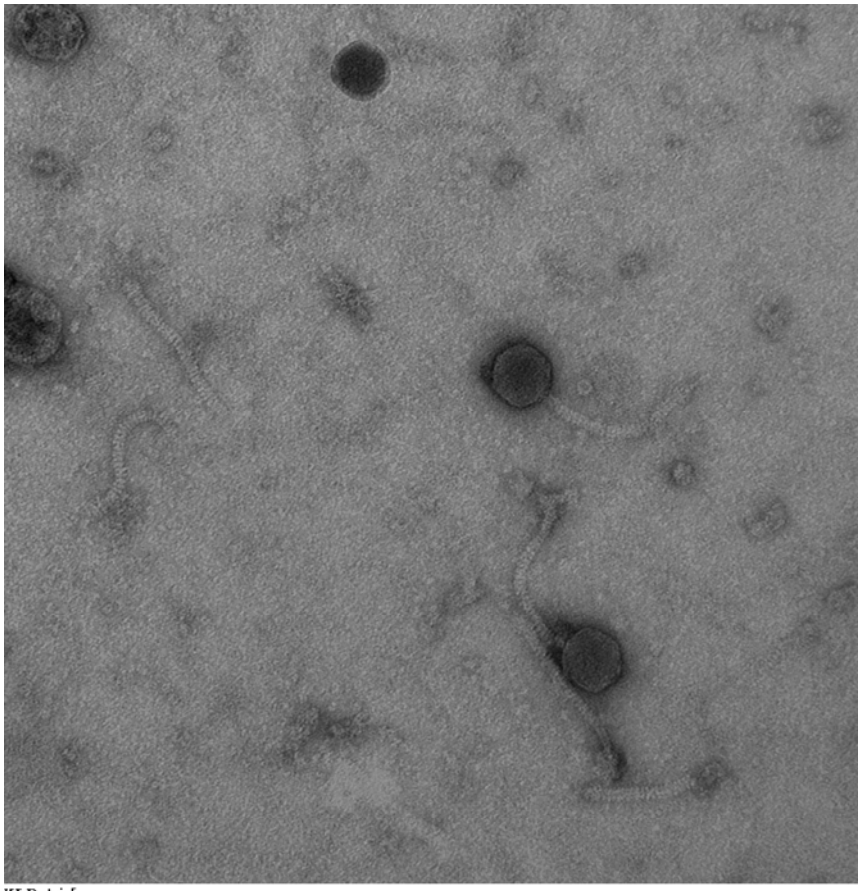
PHAGE ISOLATION (CONT'D)

- 2 ml of phage buffer added to each of the 10 plates
- Top agar and phage buffer scraped from plates and transferred to 50 mL centrifuge tube
- Agar-lysate mixture centrifuged at 4000 rpm for 15 minutes
- Supernatant filtered through 22 μm filter, collected, and titered

ELECTRON MICROSCOPY

- 10 mL of phage preparation transferred to copper EM grid
- Washed 2X with sterile water
- Stained with 1.0% uranyl acetate
- Examined with F.E.I. Morgagni Transmission Electron Microscope

EAGLE EM



100 nm

DNA ISOLATION

- 10 mL of phage lysate treated with 40 μL of nuclease mixture
- Incubated 30 minutes at 37°C and 1 hour at room temp.
- 4 mL of phage precipitant added and incubated at 4°C overnight
- Sample centrifuged at 10,000 rpm for 20 minutes

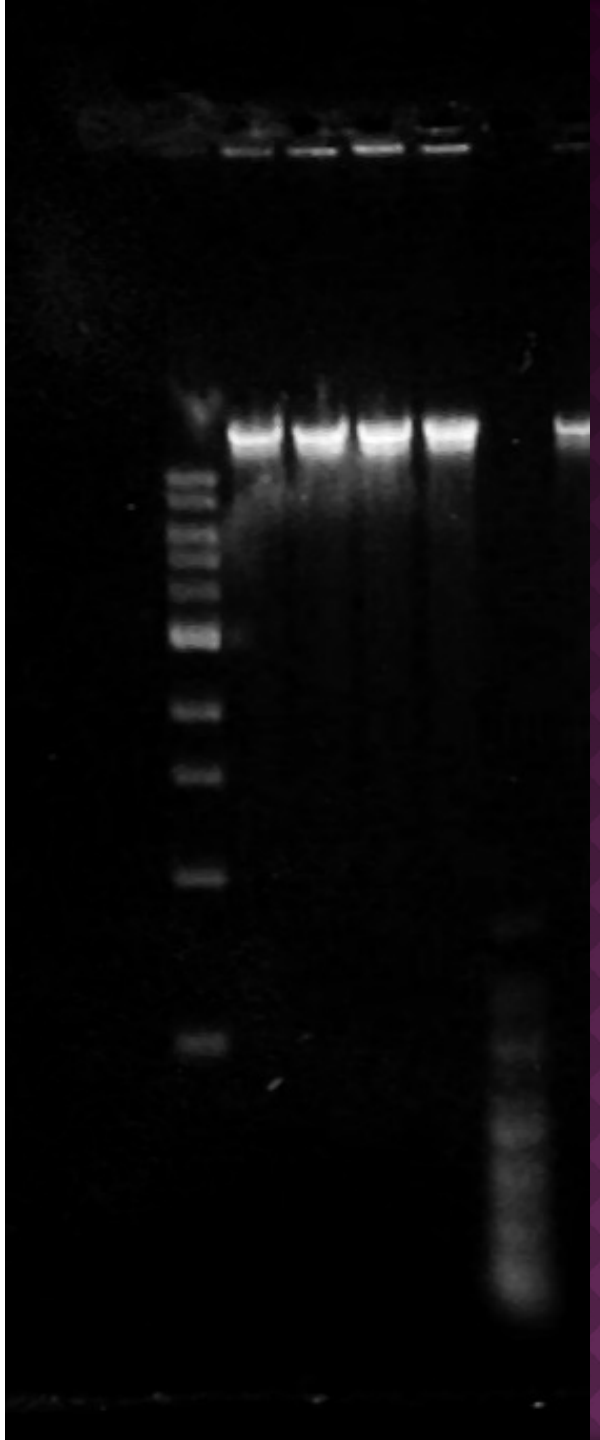
DNA ISOLATION (CONT'D)

- Pellet retained and drained
- Pellet resuspended in Promega DNA clean up resin
- Pellet subjected to Promega mini-column DNA isolation procedure
- DNA quantitated using Perkin-Elmer Lambda 40 UV/Vis spectrophotometer

RESTRICTION ANALYSIS

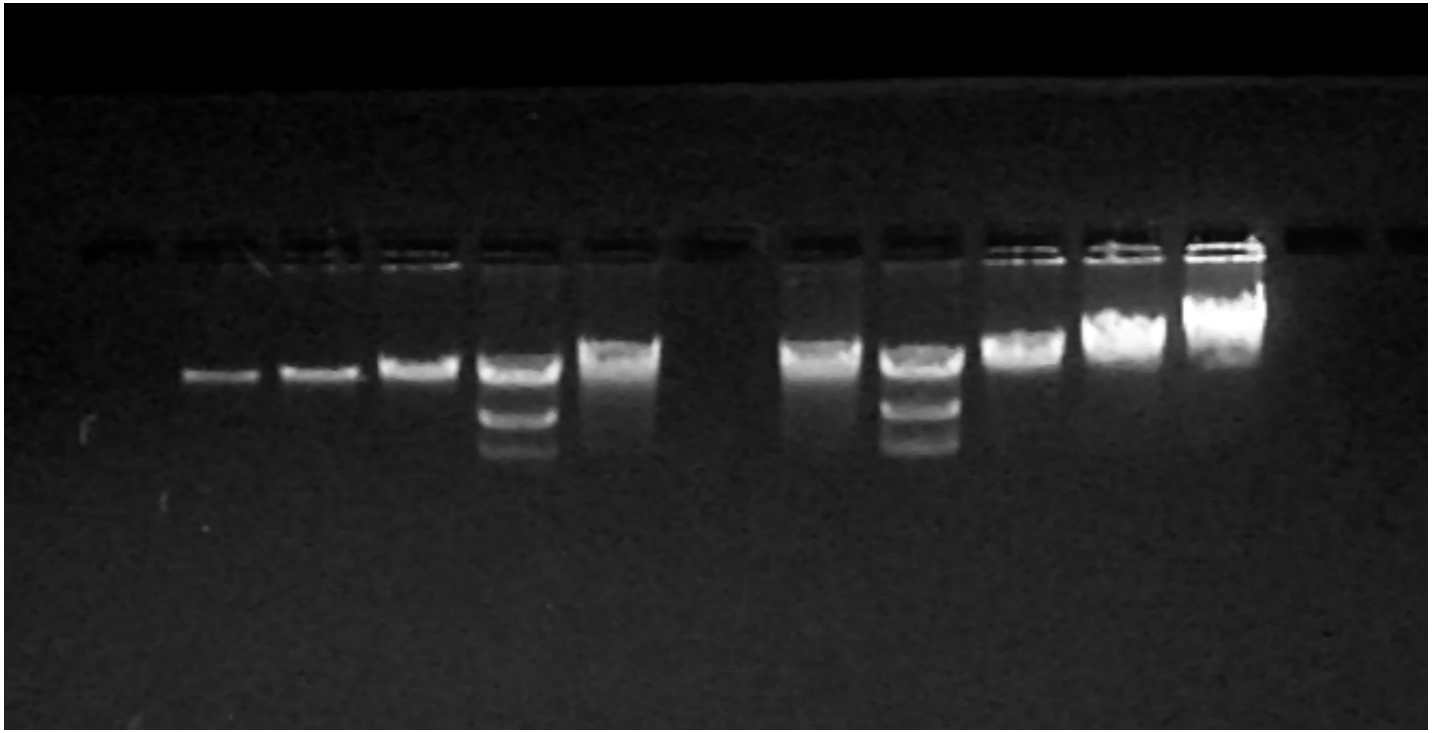
- 0.5mg of Eagle DNA mixed with restriction endonucleases
  - *Bam*H1 - *Hae*III
  - *Cla*I - *Hind*III
  - *Eco*R1
- Incubated at 37°C for 2 hours
- Run on 0.8% agarose gel for 2 hours

EAGLE RESTRICTION DIGEST



EVALUATE GENOMIC QUALITY

- 1 μL of Eagle DNA run on 1% JGI gel



SEQUENCING

- Genome sent to JGI for sequencing
- Rough sequence used to determine ambiguous region
- Primers designed using Primer 3
- Primers ordered from Fisher Scientific
- Sequencing performed using Beckman Coulter CEQ 8000 Genetic Analysis System

PCR Product after amplification with Primers

GENOME ANALYSIS

- Finished JGI sequence analyzed and annotated
- Genome length was approximately 52 kb
- The genome was divided into ~7 kb sections and pairs of students analyzed each section
- Each pair generated a poster, and lab classes compared their annotations

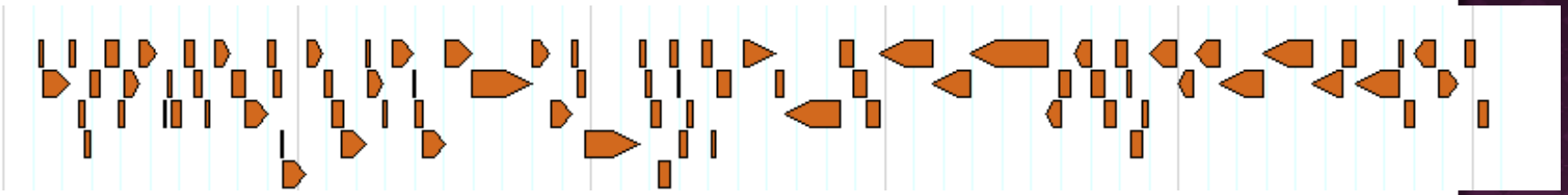
POSTER PRESENTATION

ANNOTATION

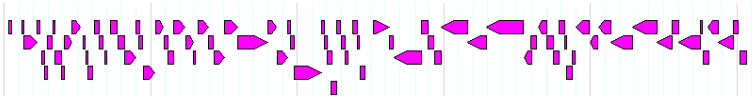
- 42 genes called and annotated
- Used BLASTx to determine matches and E values
- Used E value cutoff of 10^{-25}

GBROWSE COMPARISON

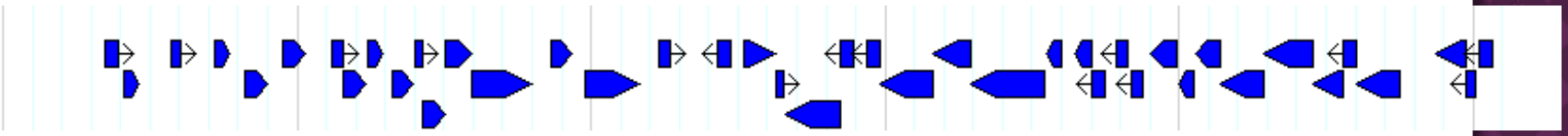
GeneMark



Glimmer



Annotated genes



PUTATIVE GENE PRODUCTS

- Tape measure protein
- Major and minor tail proteins
- Capsid protein
- DNA helicase
- DNA polymerase
- Full integrase
- Lysozyme
- Terminase

OTHER MYCOBACTERIOPHAGE MATCHES

- Vast majority in cluster A2:
  - Pukovnik
  - Bxz2
  - D29
  - L5
  - Che12

OTHER MYCOBACTERIOPHAGE MATCHES

- Several matches in cluster A1:
  - Bethlehem
  - Bxb1
  - U2
- Single matches:
  - Tweety (F1)
  - TM4 (unclustered)
  - Ms6

CONCLUSIONS

- We believe Mycobacteriophage Eagle
  - is a Siphoviridae based on the micrograph
  - is a fairly small mycobacteriophage
  - is probably not a linear genome
  - belongs to Cluster A2