Novel insight into the relationship between genomic sequence and bacteriophage morphology

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Abstract

During the 2015-2016 academic year, our Phage Hunters group at UTEP aimed to characterize novel bacteriophages infecting a laboratory strain of *Arthrobacter sp.* Arthrobacteria are typically found in humid environments; therefore, we searched for Arthrobacteriophages in two areas within our region that exhibit substantial differences in humidity: the El Paso, TX, area in the Chihuahuan desert, where forty-two soil samples were harvested, and the Lincoln National Forest in Cloudcroft, NM, where nine samples were collected. Whereas none of the samples collected in El Paso were found to contain phages, one sample collected near Cloudcroft exhibited abundant phage activity. The absence of Arthrobacteriophages in the El Paso area is in marked contrast with the high frequency of Mycobacteriophages reported in this area by our previous Phage Hunters groups. Out of the single positive sample from Cloudcroft, upon multiple rounds of plaque purification we obtained nine phage isolates, all of which were analyzed by electron microscopy and DNA restriction fingerprinting. Two morphologically distinct phage types were identified and one phage from each type, namely Piccoletto, a Myoviridae, and Nahla, a Siphoviridae, were selected for DNA sequencing and genomic annotation. According to the sequencing data obtained, Piccoletto and Nahla differed only by a 61 bp sequence that was present in both genomes but that appeared to be duplicated in Nahla. In view of such limited genomic differences between Piccoletto and Nahla, a surprising finding due to their large morphological differences, we considered it essential to further confirm the data. To this end, we first performed a second round of electron microscopy, which produced images consistent with those previously obtained. Second, we explored the genomic region containing the putative 61 bp duplication in Nahla by PCR amplification and DNA sequencing of the PCR products. In these studies, the range of templates used for PCR amplification included the DNAs used for genomic sequencing and the phages used for electron microscopy. These analyses denied the existence of any sequence difference in that region between Piccoletto and Nahla. Finally, we performed restriction analyses of genomic DNAs purified from the phages used for electron microscopy. These analyses revealed an identical DNA restriction profile for both phages. Altogether, the data obtained leads us to conclude that the dramatic difference in phage morphology between Piccoletto and Nahla must be attributed to a factor other than genomic sequence differences. We postulate that the morphological differences may be due to differentially regulated phage gene expression involving an epigenetic mechanism. The existence of two morphological stages for the *Arthrobacter* bacterial host justifies the need for two different morphologies in a phage targeting a single host.

Introduction

Arthrobacter is a gram-positive coryneform soil bacteria that is aerobic in nature and displays two distinct morphologies depending on culture age (1, 2).

Exponential Growth

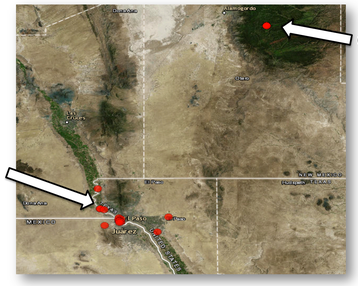
Stationary Phase

Arthrobacteriophages are bacteriophages that specifically infect Arthrobacteria. These phages are currently poorly characterized and have yet to be fully understood.

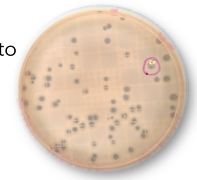
Short Retractile Tail

Long Flexible Tail

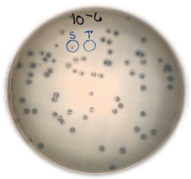
We searched for Arthrobacteriophages in two areas within our geographical region that exhibit substantial differences in humidity: The El Paso, Texas, area in the Chihuahuan desert, and the Lincoln National Forest in Cloudcroft, New Mexico.



Forty-two soil samples from the El Paso, TX, area were negative for Arthrobacteriophages. One out of nine soil samples from Cloudcroft, NM, yielded two distinct Arthrobacteriophages.



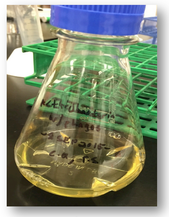
Piccoletto



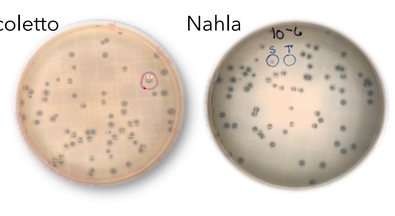
Nahla

Materials and Methods

Soil Collection

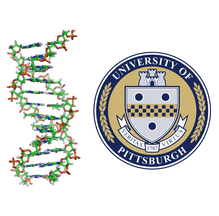


Enrichment



Isolation

Amplification



Sequencing

Genomic Analysis & PCR

Results

- Sequence data indicated that Piccoletto and Nahla differed from each other by a 61 bp repeat.
- Electron microscopy data showed substantial morphological differences.
- To confirm the data, we analyzed the region containing the 61 bp repeat by PCR and sequencing.

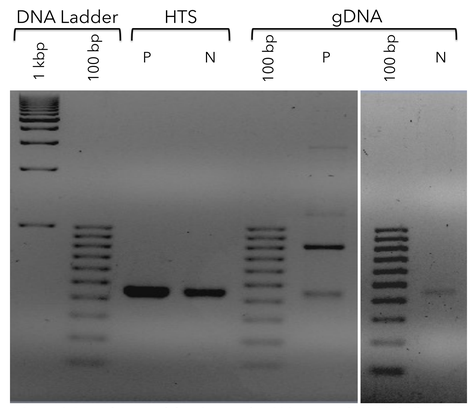


Fig. 1. PCR analyses of the region containing the putative 61 bp repeat in Nahla indicate the absence of such repeat and that the two genomes are identical in this region
PCR analyses were performed using custom designed primers to amplify the region containing the 61 bp repeat that differs between Piccoletto and Nahla. High titer phage stock (HTS) as well as genomic DNA (gDNA) for both phages were used as template DNA for individual reactions. Identical products at approximately 450 bp were obtained for all template DNAs used. 1 kbp and 100 bp indicate DNA ladders of the respective increasing length used as molecular weight markers.

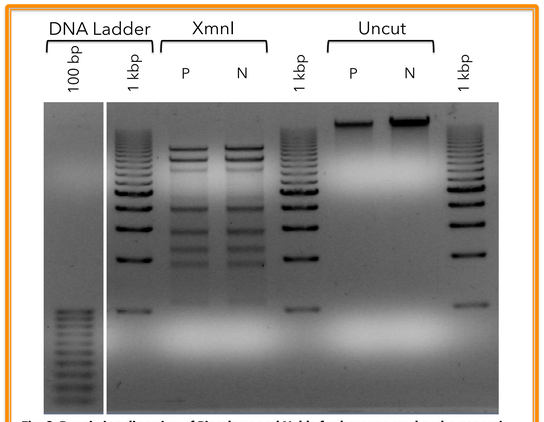


Fig. 2. Restriction digestion of Piccoletto and Nahla further support that the genomic sequences of these two phages are identical
Genomic DNA was purified from both Piccoletto and Nahla high titer phage stocks. The DNA was subjected to digestion with XmnI, which yielded the expected restriction fingerprint. The restriction profiles confirm that the two phages share identical genomic DNA. Undigested genomic DNA for each phage was included as a control.

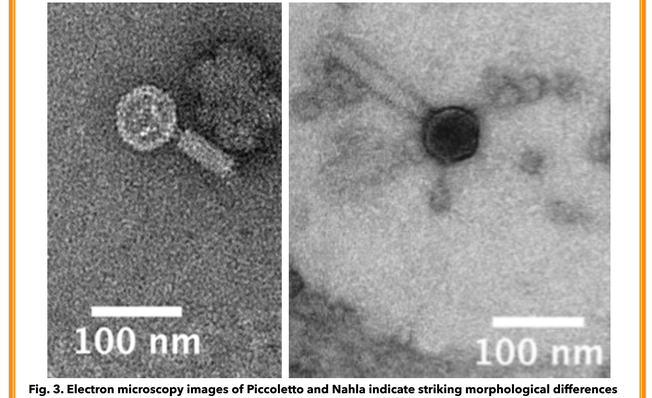


Fig. 3. Electron microscopy images of Piccoletto and Nahla indicate striking morphological differences between them
The images captured illustrate the stark differences in morphology between Piccoletto and Nahla. According to the images, Piccoletto is classified as a Myoviridae while Nahla is classified as a Siphoviridae. Surprisingly, this drastic difference was not reflected in the genomic sequences of these phages, as they were reported to be identical to one another except for the presence of the putative 61 bp repeat in Nahla. However, our PCR analyses indicated that such difference was NOT real.

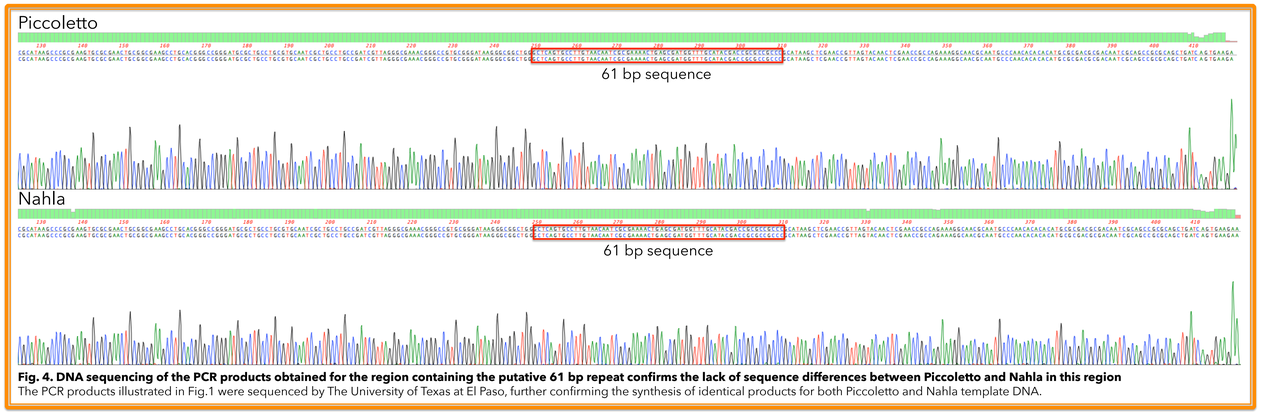


Fig. 4. DNA sequencing of the PCR products obtained for the region containing the putative 61 bp repeat confirms the lack of sequence differences between Piccoletto and Nahla in this region
The PCR products illustrated in Fig.1 were sequenced by The University of Texas at El Paso, further confirming the synthesis of identical products for both Piccoletto and Nahla template DNA.

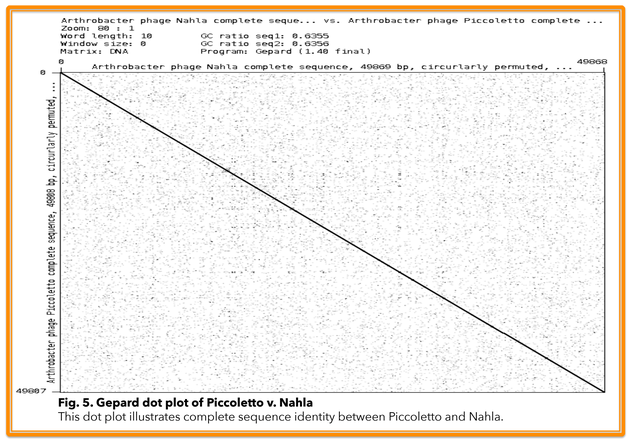


Fig. 5. Gepard dot plot of Piccoletto v. Nahla
This dot plot illustrates complete sequence identity between Piccoletto and Nahla.

Discussion

1. **The distinct morphologies** of Piccoletto and Nahla have been confirmed by two rounds of electron microscopy, demonstrating that they are morphologically different from each other.

2. **Restriction digestion and PCR analysis** have confirmed that Piccoletto and Nahla share identical genomes despite their stark morphological differences.

3. **We are led to conclude** that the dramatic difference in phage morphology between Piccoletto and Nahla must be due to a factor other than genomic sequence. We hypothesize that the morphological differences may be due to differentially regulated gene expression involving an epigenetic mechanism.

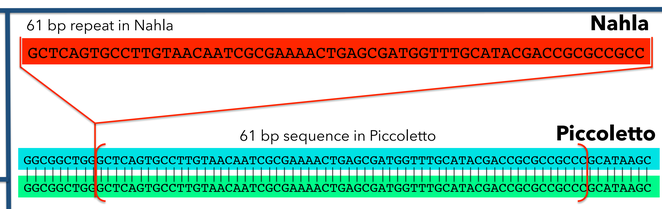


Fig. 6. Analysis of the 61 bp region
The genomic sequences generated by the University of Pittsburgh suggested that the sequences of Piccoletto and Nahla were identical except for a region of 61 bp that was repeated twice in Nahla but was present only once in Piccoletto. Our extensive analysis through restriction digest and PCR strongly indicate that such repeat is not present in Nahla, thus suggesting that it might correspond to an artifact associated to the genomic sequence assembly.

Acknowledgments

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References

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