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Sea Symposium 2022 Poster Presentation

University of North Carolina at Charlotte- Bacteriophage Discovery

In this University you are able to see and hear about the discovery portion of the sea phages which was conducted for them in the fall of 2021 but they had to begin all the way from scratch. At first they were able to understand that the discovery phage is essential to fully understand a phage as therapeutic against antimicrobial resistance and because of that this field has grown immensely. As they began they took soil in North Carolina and followed the sea phases protocol. From there these students performed the enriched isolation with m. foliorum and were able to do the procedure of modifying DNA restriction-enzyme digest. From all their phases only six of them were isolated and characterized. As they continued with the experiment they were able to get their results which detected the presence of bacteriophage inside the soil sample from the plaques. The restriction enzyme digest confirmed isolation of the phage DNA while transmission electron microscopy provided phage image.

University of California. Los Angeles- Comparative Genomic Analysis of Streptomyces Bacteriophage Clusters BE, BK, BC, AND BP

The main focus for this school was Potential reclustering, and during this experiment they were able to understand how due to the wide range of diversity the phages are clustered based on gene content similarity and the clustering threshold is 35% GCS. As they began this experiment they gave themselves a research question and goals to achieve in all of this. In there method your able to see how their main goal was to draw conclusions from the comparative genomic analyses by first allowing the streptomyces infect the phages and from there seeing all the streptomyces infecting phage gene content similarity heat map, it’s a much long process but that's how it begins. From this method they were able to see these results which they put into a five step system. The first which is the gene content comparison of representatives from all streptomyces-infecting phage clusters. Then the gene content comparison heat maps of clusters BE vs. BK and BC vs. BP, from there the Phamerator Analysis of Gene and Pham Conservation between clusters BE vs. BK and BC vs. BP phage genomes. The last two results are the evolutionary analysis of highly conserved phams between clusters BE, BK, CE, and CB, and the Phamerator and Phylogenetic Analysis of Highly conserved phams between clusters BC and BP. From their whole experiment they were able to broad the comparison of representative from all streptomyces infecting phage clusters, comparison of phages in clusters BE/BK and BC/BP, Phamerator analysis of genome architecture in cluster BE/BK and BC/BP phages, comprehensive analysis of highly conserved phams in cluster BE, BK, CE, and CB phages, and Phamerator and phylogenetic analysis of highly conserved phams in cluster BC and BP phages.

La Sierra University- Molecular Cloning and Cytotoxicity Assays of 66 Genes of Bacteriophage LeBron

In this school the students used a specific host strain that was used to isolate and further study phages Lebron is Mycobacterium smegmatis MC^2 155, which this bacterial strain is a model organism to study other mycobacterium strains, most commonly the M. tuberculosis that isn't pathogenic to humans so it makes it a useful organism to work with. Their goal with this phage Lebron was to further characterize each gene's individual toxicity to M. smegmatis in order to understand more of the gene's role within the genome. They were able to study the first sixty-six genes of the genome, in their first figure you are able to see the annotated genome map of the first sixty-six genes of phage lebron. You're able to see in this picture how if there is a known function for the gene, it is colored blue, and if there is not it is colored white and only eighteen of the genes at the moment have been assigned functions, plus ten of the genes are toxic to the host. From the ten genes that they could verify were toxic through a cytotoxicity assay only three of the genes have a previous known function, which were portal protein, Lysin A, and Tyrosine Integrase. In their results you're able to see the cytotoxicity assays of genes twenty-five and sixty-one. Gene twenty-five was a toxic gene which shows a reduction in colony size and number and in gene sixty-one which was a nontoxic gene shows no growth hindrance. In overall, the sixty-six genes successfully cloned into the p_extra01 plasmid and ten out of the sixty-six genes were found to be toxic to m. smegmatis.

Southern Connecticut State University – The annotation of a new cluster AZ phage named Tuck, and an old Cluster S phage named Lilbit

This school had done an annotation of a new cluster AZ phage named Tuck and an old cluster S phage named little bit which was assembled by these students. They were able to discover an endolysin as the first gene in Tuck's genome which is unique to all AZ ohages currently annotated other than the phage Phives which is closely related to the genome . As these students did their experiment they were able to see in their results how Lilbit had the longer genome which was 65,106 BP long, and Tuck's being 43,992 BP long. Litbit coded for 111 genes and tuck coded for 68 genes with both genomes having the evidence for forty function calls in each genome. They were able to see how their research showed that the endolysins in all of the other BLAST hits were not at the start of the genome, and how Tuck acquired this gene is unknown. These students want to do more research to determine why it is positioned where it is in the genome, as well as how it is beneficial to Tuck overall. For their next gene called Lilbit, in number gene 15 they were able to find an uncommon gene call. This gene they called annotating is replacing the gene that was originally called by Genemark on the reverse strand. With all the research they had found in the lack of coding potential in the reading frame and the coding potential of the gene called the forward strand, the decision they made was to delete the original call, and add the current gene 15 to the PECANN record. As well, they were able to see how the gene forward strand also showed 100% identity to Litbit's closest relative. Littlelaf for its gene in the same position in its genome which took them a little bit of diving in. These students while doing their experiments had interesting questions and for future class time they want to use to find out certain things they came up with while annotating their genomes.

Nyack College- Analysis of OscarSo and Cece: Pioneering Phage Isolated from micro bacteria radiodurans

These students talk about their analysis of OscarSo and Cece in which the pioneering phage is isolated using microbacterium radiodurans. Usually the microbacterium phages were isolated using m. foliorum as a host and they decided to try to isolate, identify, and characterize other phages using another microbacterium species. For the experiment they used a three step process, the first one is isolation and purification in which soil samples were enriched and the phages were isolated and purified using m. radiodurans at a 28 degree celsius. From there the next process was the host range assay which they used various bacterial species and spotten five milliliters on bacterial lawn. Lastly, they did a temperature sensitivity assay which they infected the bacterial host with phage and plated and from there they incubated at different temperatures. From all the experiments done they were able to see how 60% of the predicted genes are orphans and OscarSo is a singleton that has nine genes that are only present in Streptomyces, Arthrobacter, Gordonia, and Brevibacterium phages. The Cece is a cluster GDand it has one of the largest actinobacteriophage genomes and it has three large terminases. In the host range assay chart your able to see that oscars and cece does not infect other bacterial hosts other than which it was isolated from. As well, the temperature Sensitivity Assay the higher temperature the more plaques were observed and they were larger. For further experiments they will repeat the temperature sensitivity assay to get additional data points.

New York Institute of Technology – Determinants of Plaquing Behavior in cluster AZ phages on Arthrobacter globiformis

This school used Arthrobacter globiformis 2979 as a host to identify new actinobacteria in the fall of 2019 and 2021. They were able to find 38 AZ clusters of bacteriophages with the majority of the phages recovered from the globiformis they were using but some discovered an arthrobacter astrocyanus. They isolated 14 phages using the arthrobacter globiformis as a host from there only five of them had their genome sequenced and annotated, these five phages were five scalisa eraser genemi. Which belong to the AZ cluster and the uzumaki that belong to the au6 subcluster. They were able to receive eight arthrobacter strains for amupit and they tested which phages infected which strain. One important thing they learned was that the AZ phages were not able to infect Arthrobacter globiformis 2979 but were able to infect 2880. The uzumaki was able to infect both 2880 and 2979. The formation of AZ lysogens has already been experimentally demonstrated in Kapinos of 2021 where they made an Arthrobacter globiformis lysogen of phage powerpuff, which have superinfection immunity against phages Lego, Powerpuff, and YesChef. From there they attempted to make lysogens for Phives, Eraser, Kaylissa, Janeemi, and Uzumaki. They were able to obtain immunity assays for three of them which were Phives, kaylissa, and Janeemi. They wanted to develop an assay to reproducibly quantify the difference we observe in plaque and spot morphology. The students were able to find out how there were three different endolysin genes in cluster AZ phages. the Lysogen stability and superinfection immunity is related to phage repressor and cognate binding sites on the genome that shut down the lytic pathway.

2022 Sea Symposium Keynote Part 1

Kim Seed's Lab is studying giarial patheges fibriocalara and ICP1, In part one she spoke about the anti-phage activity and the main function that they have been studying is the PLE particles. There are multiple aspects of Ple that act synergistically to completely block ICPI progeny production. The Ple's are a dynamic but persistent part of V. cholerae's genome and in the chart you are able to see the percentage of PLe plus isolates and the total number of isolates from 1960 all the way up to 2020. In the chart you're able to see this pattern of temporal succession. In the phage fight back with nucleases you're able to see how the gaps inside of the consensus genome which are accessory regions for the phage, as well these gaps are what are changing the resistance in fibro calara. The first system Kim discovered which is responsible for helping ICP1 defend against PLe is tthe ICP1 can encode a CRISPR-Cas system targeting PLe but not all isolated ICP1 encoded CRISPR-Cas. The phages that did not encoded CRISPR-Cas did encode the Anti-PLe nuclease which was by location. THe PLE encodes a replication module composed of RepA and the compatible Ori, so every PLe needs to have a RepA protein that is compatible with its origin of replication. So if the origin changes the RepA protein has to change in order to maintain that binding event. A subset of PLes can encode a RepA protein that is mimicked by this Odn nucleus and there are other PLes that encode different functional modules. The nucleus is only available to overcome the Ples where it is mimicking that RepA protein so the other PLes are protected.