Isolation of Novel Mycobacteriophages From Soil

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Mycobacteria remain a prominent problem, especially in developing areas in South Africa and India. Mycobacterium tuberculosis causes the pulmonary disease tuberculosis (TB) and is responsible for 1.5 million deaths yearly. Leprosy, caused by Mycobacterium leprae, has also made a recent resurgence in Florida, USA. The long growth time of TB, 2-3 weeks to form colonies, prolongs experimentation and diagnosis time. Mycobacterium smegmatis mc2 155, a strain of M. smegmatis, displays the EPT (efficient plasmid transformation) phenotype, and was used in this study as a safe and fast growing (2-3d for colonies) substitute for M. tuberculosis. I screened forty soil samples from locations including the U.S.A., India, Mexico and Guatemala for phage presence by means of a plaque assay with M. smegmatis mc2155 and an attenuated M. tuberculosis strain (used by mentor). Environmental samples were filtered and plated with M. smegmatis mc2 155 for 2-3d, following which plaques were picked and the phage stock was propagated. A Guatemalan soil sample contained a phage which was shown to infect M. smegmatis mc2 155 and not infect M. tuberculosis. An analytical restriction digest was performed with enzymes EcoR1, EcoR5, Nhe1, Pst1 and Xho1, however a low DNA signal was observed, likely due to excessive dilution of extracted DNA. Next steps include development of shuttle phasmids, which are recombinant phages that replicate as plasmids in Escherichia coli and phages in mycobacteria, which allow for the efficient production of phage DNA. Significant progress has been made towards engineering shuttle phasmids with the phage isolated from Guatemala as well as other phages.

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