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The isolation of 205 bacteriophages on Microbacterium foliorum: Some struggles and some successes

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With the hope of discovering new bacteriophages and expanding our understanding of phage diversity, we searched for phages that could grow on *Microbacterium foliorum* NRRL B-24224 SEA in the Phage Discovery Lab course, BIOL 105L, at Gonzaga University during the academic year 2017-2018. We purified and characterized 205 phage isolates from enrichment cultures of soil, grass, acorn, moss, bark and water samples. The phages were propagated at room temperature (22-25 deg C) during isolation, purification, and amplification. At this temperature, lawns and plaques could be observed after 24 hours, but plaques continued to grow in size over the next 3-4 days. Compared to previous students' experiences with *M. smegmatis* phages, we found that a higher percentage of isolates were difficult to work with. A number of us found it difficult to prepare a lysate with a titer greater than 108 pfu/ml, a number of phages appeared distorted in TEM images, and a large percentage of lysates produced DNA that appeared to be fragmented or degraded. We report here on our experiences with these experiments, and our efforts to troubleshoot working with *M. foliorum* phages.