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A jumbo discovery: Omphalos is the largest actinobacteriophage found to date

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To expand knowledge of bacteriophage diversity in the phylum Actinobacteria, we searched for phages that could grow on the host *Microbacterium testaceum NRRL B-59317*. *M. testaceum* isolates typically live on and in plants without causing disease symptoms. *M. testaceum NRRL B-59317*, however, was found in a NASA clean room during assembly of the Mars lander, Phoenix. Phage Omphalos was isolated from soil sampled from a pumpkin garden and enriched in medium containing *M. testaceum*. Omphalos produced large, turbid plaques at 30 deg C with an irregular, multi-ringed appearance. Clear plaques routinely appeared on these plates, but we have not propagated them to test heritability. At 37 deg C, all plaques were clear. TEM showed that Omphalos is a non-contractile tailed phage (capsid width, 100 nm; tail length, 250 nm). The Omphalos genome is 235,841 bp in length, which places it in the group of >200 kb phages known as giant or jumbo phages. It is the first jumbo phage discovered in the Actinobacteria. The genome ends contain long, direct terminal repeats of 18,632 bp. The GC content of Omphalos is 34%, whereas the *M. testaceum* genome is 70% GC. Omphalos contains 318 predicted protein-coding genes, 33 of which are duplicated in the direct terminal repeats, and two tRNA genes. The genome is a singleton among actinobacteriophages, but is distantly related to a clade of extrachromosomal DNAs in bacterial genomes which are annotated as large plasmids, as well as the singleton *Bacillus* phage bp0305Phi836 and the singleton *Acinetobacter* phage vB-AbaM-ME3. In total we identified 37 supposedly bacterial contigs or plasmids related to Omphalos, all of which were approximately the size of jumbo phage genomes (150-800 kb) and contained terminase, portal, and capsid proteins. Most of these were found in the genomes of species in the phylum Firmicutes, a phylum distantly related to the Actinobacteria which has convergently evolved a gram-positive cell wall morphology. The DNA sequences most closely related to Omphalos all came from bacteria in the genus *Bacillus*, and shared on average 127 genes with Omphalos (as determined by blastp similarity). Many shared genes are syntenic between Omphalos and the *Bacillus* sequences. Regions of 80-90 kb containing structural genes encoding terminase, portal, major capsid protein, and tail proteins are in nearly identical order. The strongest alignments show 35-75% amino acid identity across the length of the proteins. The major capsid proteins, for example, share 71% aa identity. To the right of this region, genes with functions related to DNA replication, DNA metabolism, and recombination are shared between Omphalos and the *Bacillus* sequences, but gene order is not as well conserved.