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Complete Genome Sequences of *Streptomyces* Bacteriophages Annihilus, TonyStarch, Thiqqums, CricKo, ClubPenguin, RosaAsantewaa, and PherryCruz

Yvette Genie Park,^a Gillian Faith McCarthy,^a Hadeeqa Mustafa,^a Gemma M. Feild,^a Snigdha Puram,^a Hager Aly Younes,^a Danyah Imam,^a 2021 UMBC Phage Hunters, STEM BUILD at UMBC Cohort 5,  Ivan Erill,^a  Steven M. Caruso^a

^aDepartment of Biological Sciences, University of Maryland Baltimore County, Baltimore, Maryland, USA

ABSTRACT Seven siphoviruses were isolated from soil using *Streptomyces* hosts. Their genome sequences ranged from 42,730 to 57,624 bp long and had a GC content of approximately 60%. Based on their gene content similarity to actinobacteriophages, all seven phages were assigned to cluster BI. For several of these phages, multiple ribosomal frameshifts were identified.

Streptomyces species are well known for the production of antibiotics and other bioactive compounds. Here, we report on seven bacteriophages isolated from soil samples on two members of the genus, *Streptomyces scabiei* RL-34 (ATCC 49173), a plant pathogen that causes potato scab disease (1), and *Streptomyces mirabilis* NRRL-2400 (ARS), a species able to grow in soils containing heavy metals (2), using standard methods (3) (Table 1). Briefly, soil samples were washed in phage buffer (10 mM Tris [pH 7.5], 10 mM MgSO₄, 1 mM CaCl₂, 68.5 mM NaCl), and the wash was collected by centrifugation and filtration (0.22-μm filter). The filtrate was then plated in tryptic soy soft agar (BD), with either *S. scabiei* or *S. mirabilis* overlaid on nutrient agar (BD Difco) supplemented with 10 mM MgCl₂, 8 mM Ca(NO₃)₂, and 0.5% glucose, and incubated at 30°C for 1 to 2 days to yield bacteriophages Annihilus, TonyStarch, Thiqqums, CricKo, ClubPenguin, and RosaAsantewaa. For one soil sample, the filtrate was first inoculated with *S. scabiei* RL-34 and incubated with shaking for 24 h at 30°C; then, the culture was filtered and plated in soft agar with *S. scabiei* yielding phage PherryCruz. All phages were purified with a minimum of three rounds of plating. Negative stain transmission electron microscopy revealed all seven bacteriophages to be siphoviruses (4) (Fig. 1A). The particle capsid and tail measurements are provided in Table 1.

Genomic DNA of all seven bacteriophages was isolated from crude lysate and purified using a Promega Wizard DNA cleanup system, prepared for sequencing using the NEB Ultra II library kit, and sequenced at the Pittsburgh Bacteriophage Institute using the Illumina MiSeq platform (v3 reagents), producing over 100,000 150-base single-end reads for each phage (Table 1). The raw reads were assembled using Newbler v2.9. Quality control was performed using Consed v29 (5). The genome ends were identified by comparison to similar phages with known ends and confirmed by read start buildups. Based on the gene content similarity, all seven phages were assigned using PhagesDB to actinobacteriophage cluster BI (6–8). The sequencing data, genome characteristics, and cluster assignments are provided in Table 1.

Genome annotation was completed using DNA Master v5.23.6 (9) embedded with Glimmer v3.02b (10), GeneMark v4.28 (11), Phamerator v.Actino_draft 463 (12), NCBI blastp v2.13.0 (13), and HHpred v57c87 (14). The phages were found to have from 55 (RosaAsantewaa) to 94 (CricKo, Thiqqums) protein coding genes, of which an average 32% were assigned functions. No tRNA coding genes were identified using tRNAscan-SE v2.0 (15) or Aragorn v1.2.41 (16).

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Address correspondence to Steven M. Caruso, scaruso@umbc.edu.

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TABLE 1 Summary properties of the analyzed B1 *Streptomyces* bacteriophages

Phage name	Collection location	Host	Particle head diam \pm SD, tail length \pm SD (nm); no. of particles	Total no. of 150-bp reads	Cluster	Genome length (bp)	GC content (%)	3' single-stranded end sequence	GenBank accession no.	SRA accession no.
TonyStarch	Ellicott City, MD (39,262 N, 76.818 W)	<i>S. mirabilis</i>	55 \pm 1, 285 \pm 8; 3	972,262	Bl1	55,469	59.6	5'-CGCCCGCCT-3'	ON108646	SRX14485098
Annihilus	Glen Burnie, MD (39.133021 N, 76.628615 W)	<i>S. scabiei</i>	52 \pm 2, 238 \pm 24; 4	1,895,132	Bl2	43,562	61.2	5'-CGCCCGCCT-3'	ON081336	SRX14443513
PherryCruz	Halethorpe, MD (39,257417 N, 76.704278 W)	<i>S. scabiei</i>	52 \pm 2, 228 \pm 8; 8	1,005,742	Bl2	43,736	61.0	5'-CGCCCGCCT-3'	MK686070	SRX14814648
RosaAsantewaa	Accra, Ghana (5.6052560 N, 0.1733080 W)	<i>S. mirabilis</i>	47 \pm 4, 234 \pm 15; 5	832,774	Bl2	42,730	58.8	5'-CGCCCGCCT-3'	MK686072	SRX14814650
Cricko	Baltimore, MD (39,25366 N, 76.71331 W)	<i>S. scabiei</i>	62 \pm 3, 271 \pm 6; 4	1,086,915	Bl4	57,623	58.1	5'-CGCCCGCCT-3'	MT310854	SRX14814644
Thiqqums	Catonsville, MD (39,2585 N, 76.7131 W)	<i>S. scabiei</i>	50, 200; 1	932,673	Bl4	57,624	58.1	5'-CGCCCGCCT-3'	MT657340	SRX14814651
ClubPenguin	Millersville, MD (39,096152 N, 76.573399 W)	<i>S. mirabilis</i>	61 \pm 2, 264 \pm 16; 5	913,162	Bl7	56,205	59.0	5'-CGCCCGCCT-3'	MT310852	SRX14814643



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Members of the 2021–2022 UMBC Phage Hunters class are listed at <https://phages.umbc.edu/home/class-lists/2021-22/>. Members of STEM BUILD at UMBC Cohort 5 are listed at <https://phages.umbc.edu/home/class-lists/stem-build/cohort-5-summer-2020/>.

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