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Genome Sequences of Nine Bacteriophages Infecting *Streptomyces bicolor*: Dexers, Jhitchelle, Kaine, Marav, Pavo, Provolone, SheRa, Speedwell, and Sudan

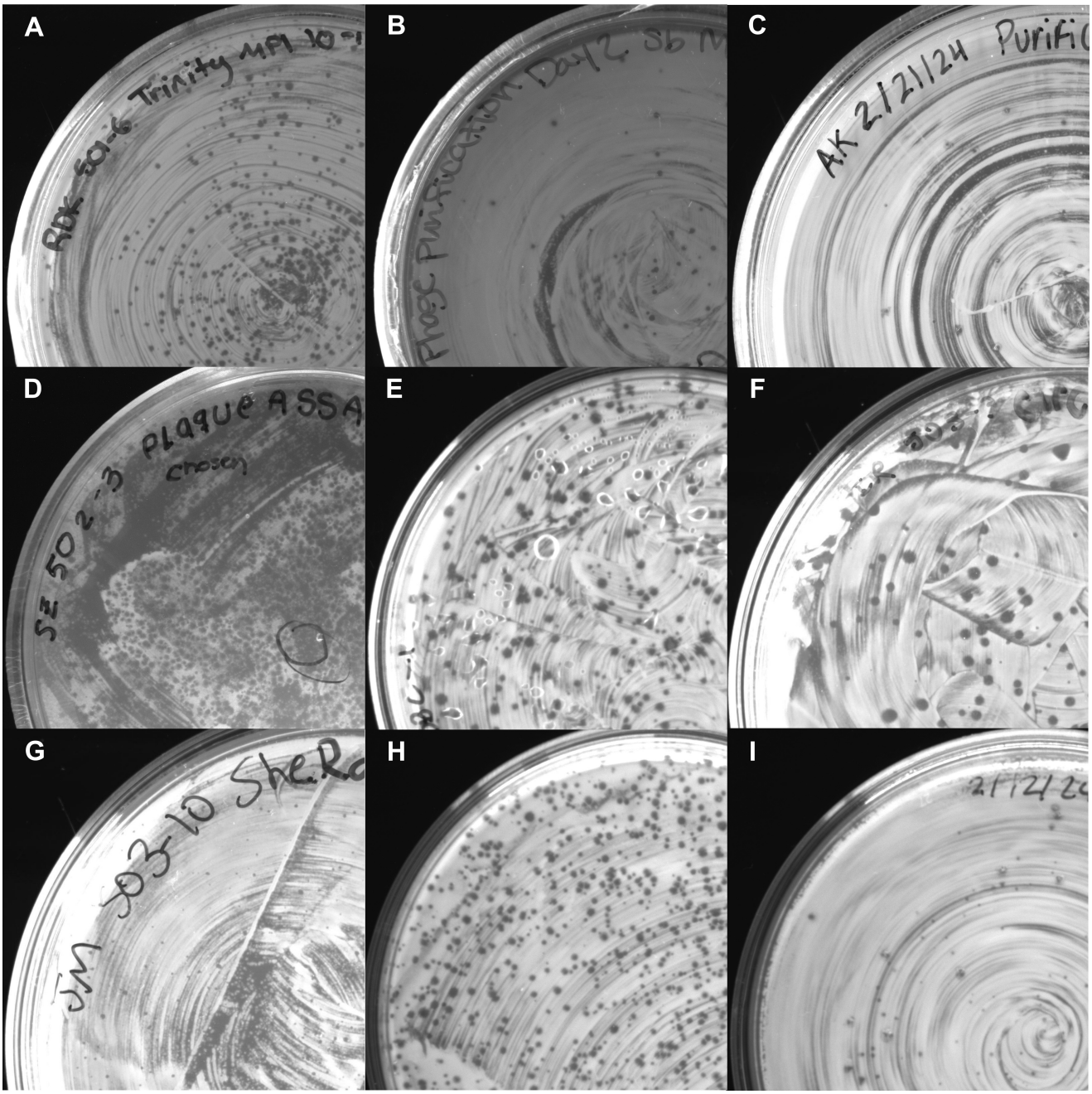
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Abstract

Bacteriophages Dexers, Jhitchelle, Kaine, Marav, Pavo, Provolone, SheRa, Speedwell, and Sudan were isolated on *Streptomyces bicolor* NRRL B-3897. These bacteriophages have genomes sizes spanning 43–50 kbp with 59–82 genes and assigned to clusters BD2, BD3, and BI1. Only one, Marav, encodes a tRNA. These are the first bacteriophage isolates reported for this species of *Streptomyces*.



Phage name	Sampling location (City, State, GPS)	Genome length (bp)	GC content (%)	Sequencing Fold coverage	Number of Reads	No. of Genes (# with predicted function	No. of tRNA genes
Dexers	Denton, TX,	48907	66.00%	2435	830,832	74(36)	0
	(33.17704 N, 97.12435 W)						
Jhitchelle	Brunswick, GA,	48149	65.60%	196	93,578	73(38)	0
	(31.218408 N, 81.520637 W)						
Kaine	Little Elm, TX,	48825	65.80%	5753	2,905,592	76(36)	0
	(33.15595 N, 96.94897 W)						
Marav	Aubrey, TX,	50932	67.60%	2126	759,591	82(38)	1

	(33.253190 N, 96.951315 W)						
Pavo	Flower Mound, TX,	48901	65.90%	7752	3,996,167	77(37)	0
	(33.0276 N, 97.0944 W)						
Provolone	Denton, TX,	49042	65.90%	1901	649,157	77(36)	0
	(33.18371 N, 97.10242 W)						
SheRa	Denton, TX,	43596	60.90%	2377	727,854	59(21)	0
	(33.207858 N, 97.15029 W)						
Speedwell	Flower Mound, TX,	49789	65.90%	5727	2,983,132	78(39)	0
	(33.05572 N, 97.09381 W)						
Sudan	Denton, TX,	48808	65.90%	402	197,278	76(38)	0
	(33.21048 N, 97.151563 W)						

Figure 1. Plaque Morphology:

Plaque morphology on lawns of *Streptomyces bicolor* for (A) Dexers, (B) Jhitchelle, (C) Kaine,(D) Marav, (E) Pavo, (F) Provolone, (G) SheRa, (H) Speedwell, and (I) Sudan shown on standard 100mm diameter plates. Table 1: Bacteriophage genome characteristics.

Description

The genus *Streptomyces* is diverse, contains many industrially-important species, and is best known for the large number of antibiotics produced by its members. Over 400 *Streptomyces* bacteriophage genomes have been sequenced and grouped into 21 clusters (BA–BT,JN), with additional unclustered bacteriophages, in the Actinobacteriophage database (https://phagesdb.org/). Contributing to this diversity, nine bacteriophages—Dexers, Jhitchelle, Kaine, Marav, Pavo, Provolone, SheRa, Speedwell, and Sudan—were isolated from soil samples collected in Texas and Georgia using *Streptomyces bicolor* NRRL B-3897 as the host (Table 1). These bacteriophages add to the growing catalog of actinobacteriophages and provide further insight into genomic diversity in bacteriophages infecting *Streptomyces*.

Isolation was performed using an enrichment method. Briefly, soil samples were suspended in nutrient broth, inoculated with *S. bicolor*, and the mixture incubated while shaking at 30°C for two days (Poxleitner et al., 2017; Zorawik et al., 2024). Following incubation, samples were centrifuged and filtered through 0.22-µm filters, then spread plated on nutrient agar supplemented with $MgCl_2$, $Ca(NO_3)_2$, and glucose. Individual plaques were purified through a minimum of three rounds of serial dilution and replating, and high-titer lysates (>1 × 10^8 PFU/mL) were obtained through multi-plate amplification. All isolates produced clear, circular plaques. Plaque diameters ranged from 0.25 to 2 mm (Figure 1).

DNA extraction was performed using the Promega Wizard DNA Cleanup System. Genomic DNA was sequenced at the University of Pittsburgh Bacteriophage Institute (Dexers, Kaine, Marav, Pavo, Provolone, SheRa, Speedwell) or the University of North Texas Genomics Center (Jhitchelle, Sudan) using Illumina sequencing. Sequencing libraries were prepared with the NEB Ultra II Library Kit, and 75-base single-end reads (Jhitchelle, Sudan), 100-base single-end reads (Kaine, Pavo, Speedwell) or 150-base single-end reads (Dexers, Marav, Provolone, SheRa) were generated on an Illumina MiSeq instrument using v3 reagents. Reads were assembled into single contigs following the method described by Russell (2018) using Newbler v2.9 (Miller et al., 2010) and Consed v29 (Gordon et al., 1998). Sequencing outcomes and genome characteristics are summarized in Table 1. All nine bacteriophages possess linear genomes with 3′ sticky overhang ends. Marav has 11-base overhangs (CGCCACGTCTT); Dexers, Jhitchelle, Kaine, Pavo, Provolone, Speedwell, and Sudan have 11-base overhangs (CGGTACGTGAT); and SheRa has a 9-base overhangs (CGCCGCCCT).

Annotation was conducted using multiple bioinformatics tools with default settings used for all software. Tools included DNA Master (Pope & Jacobs-Sera, 2018) for genome visualization, auto-annotation, and manual annotation refinement, PECAAN (Rinehart et al., 2016) for evidence integration and guided annotation support, Glimmer v.3.02 (Delcher et al., 2007) and GeneMark v.2.5 (Besemer & Borodovsky, 2005) for predicting protein-coding genes, Phamerator (Actiono_draft database v578) (Cresawn et al., 2011) for comparing gene content and synteny among related phages,

BLASTp and NCBI BLAST v.2.13 (Altschul et al., 1990) for identifying similarity to known genes, HHpred (using databases: PDB_mmCIF70, NCBI_Conserved_Domains, and Pfam- v.36) (Söding et al., 2005) for detecting remote protein homology and predicting function, Aragorn v.1.2.41 (Laslett & Canback, 2004) and tRNAscan-SE v. 2.0.12 (Lowe & Chan, 2016) for identifying tRNA genes, and DeepTMHMM v.1.0.44 (Hallgren et al., 2022) for predicting transmembrane domains.

Based on shared gene content similarity greater than 35%, Marav was assigned to subcluster BD2; Dexers, Jhitchelle, Kaine, Pavo, Provolone, Speedwell, and Sudan were assigned to subcluster BD3, and SheRa to subcluster BI2. The GC content for all nine bacteriophages was over 60%, consistent with other *Streptomyces* bacteriophages. Functionally, the cluster BD bacteriophages isolated in this study encode a putative serine integrase from a gene phamily commonly found in other cluster BD phages, suggesting a temperate life cycle. However, SheRa lacks an identifiable integrase and, like other BI2 phages, is likely lytic. Marav was the only genome in this study to contain a tRNA gene, encoding for phenylalanine [Phe(GAA)]. About a third of all annotated members of subcluster BD2 also contain a single tRNA. With the isolation of the bacteriophages in this study, *S. bicolor* becomes the fourteenth different species on which cluster BD bacteriophages have been isolated and the tenth species for BI bacteriophages. While these genomes show similarity with genomes isolated on other *Streptomyces* host species and fit within existing actinobacteriophage clusters, they also exhibit new genetic diversity. Six of the nine isolates contain genes that do not fit into existing gene phamilies (1 each in Jhitchelle [gp49], Kaine [gp51], Marav [gp7], Pavo [gp53], and Sudan [gp4]; 3 in Speedwell [gp9, gp10, gp12]).

Nucleotide Sequence Accession Numbers

The GenBank and SRA accession numbers for the phages in this study are: Dexers (Accession No. OQ995439, SRA No. SRX21368059), Jhitchelle (Accession No. PZ319869, SRA No. SRX33201634), Kaine (Accession No. PQ723753, SRA No. SRX28484020), Marav (Accession No. OQ995441, SRA No. SRX20165769), Pavo (Accession No. PQ723757, SRA No. SRX28484025), Provolone (Accession No. OQ995429, SRA No. SRX21368081), SheRa (Accession No. OQ995437, SRA No. SRX21368085), Speedwell (Accession No. PQ723754, SRA No. SRX28484031), and Sudan (Accession No. PZ319862, SRA No. SRX33201643).

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