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ANNOTATED SEQUENCE RECORD

Sequencing, genome analysis and host range of a novel *Ralstonia* phage, RsoP1EGY, isolated in Egypt

Abdelmonim Ali Ahmad[1,2] · Kamel M. Elhalag[3,4] · Hardian Susilo Addy[1,5] · Mohamed A. Nasr-Eldin[6] · Ahmed S. Hussien[3,7] · Qi Huang[1]

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Abstract

A novel *Ralstonia* phage was isolated from soil in Egypt. It was designated *Ralstonia* phage RsoP1EGY using our phage identifier naming approach to reflect the phage's bacterial host species, characteristics and origin. When tested, this phage specifically infected only race 3 biovar 2 phylotype IIB sequevar 1, and not non-race 3 biovar 2 strains of *Ralstonia solanacearum*. The phage has an icosahedral capsid of 60 ± 5 nm in diameter with a short tail of 15 ± 5 nm in length, typical of a podovirus. The genome of RsoP1EGY is 41,297 bp in size, containing 50 open reading frames, with no significant sequence identity to any other reported *R. solanacearum* or non-*Ralstonia* phages, except to the recently deposited but unreported and unclassified *Ralstonia* phage DU_RP_I. RsoP1EGY is the first sequenced and characterized *R. solanacearum* phage isolated in Egypt.

Potato is the second most important vegetable crop next to tomato in Egypt. Egypt also ranks among the world's top potato exporters, mainly for markets in Europe. Potato brown rot caused by cool-temperature-adapted race 3 biovar 2 (r3b2) strains of *Ralstonia solanacearum* is a serious problem in Egypt that affects potato production and exportation. As a result, *R. solanacearum* is a quarantine pathogen with zero tolerance in Egypt. The r3b2 strains of *R. solanacearum* are also quarantined pathogens in Europe and select agents in the United States [1]. Bacteriophages that specifically infect *R. solanacearum* have recently been isolated from Japan, Thailand, Korea and the United States, and efforts have been made to determine their potential as biocontrol agents for *R. solanaceraum* [2–5].

In this study, a bacteriophage was isolated from the soil of an *R. solanacearum*-infested potato field in 2017 in Al Qalyubia governorate of Egypt using *R. solanacearum* strain K3 as the host, based on the method of Ahmad et al. [6]. The phage was purified from a single-plaque isolate, and a phage susceptibility test was performed (Table 1) using a spot test followed by a plaque assay as described by Ahmad et al. [2]. *R. solanacearum* was cultured as described [2], and strains UW550 and UW552 were routinely used as hosts for propagation of the phage. A total of one liter of bacterial culture (ten 100-ml cultures) was grown in order to obtain a sufficient amount of the phage particles. When the bacterial culture reached the absorbance of 0.2 at 600 nm, phage suspension was added at a

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✉ Qi Huang
qi.huang@ars.usda.gov

1 Floral and Nursery Plants Research Unit, United States National Arboretum, Agricultural Research Service, United States Department of Agriculture, Beltsville, MD, USA

2 Department of Plant Pathology, Faculty of Agriculture, Minia University, El-minia, Egypt

3 Bacterial Diseases Research Department, Plant Pathology Research Institute, Agricultural Research Center (ARC), Giza, Egypt

4 Potato Brown Rot Project, Ministry of Agriculture, Dokki, Giza, Egypt

5 Department of Plant Protection, Faculty of Agriculture, University of Jember, Jember, Indonesia

6 Botany Department, Faculty of Science, Benha University, Benha, Qalyubiya Governorate, Egypt

7 Central Administration of Plant Quarantine, Dokki, Giza, Egypt

Table 1 Susceptibility of *Ralstonia solanacearum* strains to *Ralstonia* phage RsoP1EGY

Ralstonia solancearum (Phylotype-sequevar/biovar)	Origin	Source	RsoP1EGY[a]
Race 3 biovar 2 strains			
UW257 (IIB-1)	Costa Rica	C. Allen, USA	+
UW344 (IIB-1)	Brazil	C. Allen, USA	+
UW550 (IIB-1)	Netherlands	C. Allen, USA	+
UW551 (IIB-1)	Kenya	C. Allen, USA	+
UW552 (IIB-1)	Guatemala	C. Allen, USA	+
RUN035 (IIB-1)	Netherlands	P. Prior, France	+
IVIA1602.1 (IIB-1)	Spain	M. M. López, Spain	+
K3 (IIB-1)	Egypt	This study	+
K10 (IIB-1)	Egypt	This study	+
K13 (IIB-1)	Egypt	This study	+
Non-race 3 biovar 2 strains			
Rs5 (IIA-7/1)	USA	J. Jones, USA	–
Rs121 (I/1)	USA	J. Jones, USA	–
AW1)II-7/1)	USA	C. Allen, USA	–
K60 (IIA-7/1)	USA	C. Allen, USA	–
UW119 (I/3)	Costa Rica	C. Allen, USA	–
GM1000 (I-18/3)	French Guiana	P. Prior, France	–
RUN302 (IIB-4/1)	Brazil	P. Prior, France	–
Pss530 (I/3)	Taiwan	J. F. Wang, Taiwan	–
Pss51 (I-15, I/4)	Taiwan	J. F. Wang, Taiwan	–
DT3 (I/3)	Indonesia	H. Addy, Indonesia	–

[a]Susceptibility of *R. solanacearum* strains to phage RsoP1EGY was determined by spot test followed by plaque assay. It is shown as susceptible (+) when clear plaques were observed or resistant (–) when no plaques were observed

multiplicity of infection of 0.1. After further growth for approximately 20 h, bacterial cells were removed, and phage particles were purified for morphological characterization under a Hitachi 7700 transmission electron microscope and for DNA extraction as described by Ahmad et al. [2], except that the supernatant was filtered through a 0.45-µm membrane before ultracentrifugation.

The phage has an icosahedral capsid of 60 ± 5 nm in diameter with a short tail of 15 ± 5 nm in length (Fig. 1A), the typical morphology of members of the family *Podoviridae*. We named the phage *Ralstonia* phage RsoP1EGY, since it is the first *R. solancearum*-infecting bacteriophage belonging to the family *Podoviridae*, and it was isolated in Egypt. Our naming of the phage was based on the informal guide by Adriaenessens and Brister [7] (i.e., a tripartite phage name – *Ralstonia* phage RsoP1EGY) and the scheme proposed by Kropinski et al. [8] (i.e. Rso for *R. solanacearum* and P for *Podoviridae*). Additionally, our name took the sequential number of the phage we isolated in the same virus family into consideration (i.e., 1 for the first isolated phage), as well as where the phage was originally isolated, by including a three-letter country code (i.e., EGY for Egypt). This naming approach aims to not only make the identifier name unique but also to make it systematic and meaningful to other researchers regarding the phage's bacterial host species, characteristics and origin.

Genomic DNA of phage RsoP1EGY was extracted from purified phage particles (described above) using either a Phage DNA Isolation Kit (Norgen Biotek Corp, Canada), or the phenol-chloroform method [9]. The phage DNA was sequenced and the genome sequence assembled commercially by SeqMatic LLC (Fremont, California). Potential open reading frames (ORFs) larger than 40 amino acids were identified using PHAST (http://phast.wishartlab.com) and GeneMark [10]. Homology searches for each identified ORF were performed using BLAST/PSI-BLAST [11] against NCBI's protein databases [12, 13]. An e-value threshold of e^{-4} or less was used for two proteins to be considered a match.

The genome of phage RsoP1EGY is a double-stranded DNA of 41,297 bp in length, with a G+C content of 59% (GenBank accession no. MG711516). When the complete genome sequence of phage RsoP1EGY was used as a query to search NCBI's nucleotide collection by BLASTn, the phage genome has no significant sequence identity to any of the previously published *R. solanacearum* phages, except that it shared 99% nucleotide sequence identity with 95% coverage with a recently deposited but unpublished

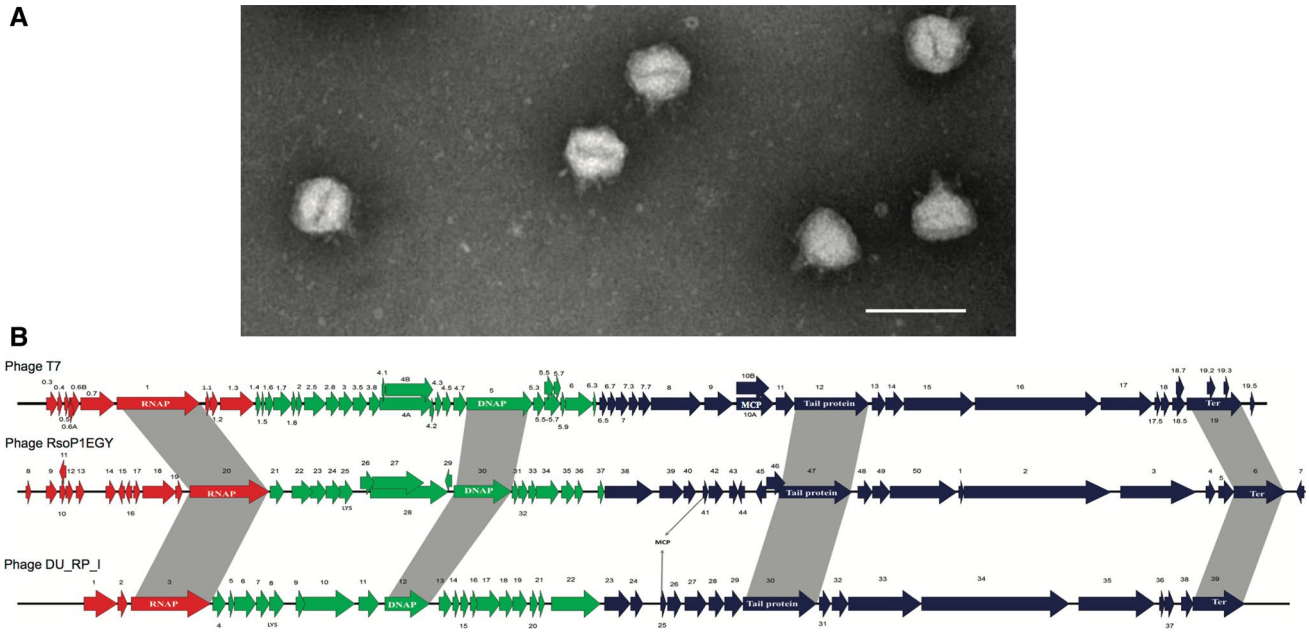

Fig. 1 Electron micrograph of *Ralstonia* phage RsoP1EGY virions (**A**) and genome organization of phages RsoP1EGY and DU_RP_I, with reference to the *Enterobacteria* phage T7 (**B**). The scale represents 100 nm. Arrows represent the size and direction of transcription of ORFs. Functional gene clusters I for early genes, II for DNA metabolism, and III for virion structure and assembly of phages RsoP1EGY and DU_RP_I are assigned according to the typical podovirus genome represented by the T7 phage [12] and marked in red, green and blue, respectively. Numbers are annotated ORFs in each phage. RNAP, RNA polymerase; DNAP, DNA polymerase; MCP, major capsid protein; Ter, terminase

"*Ralstonia* phage DU_RP_I" (GenBank accession no. MF979559.1; Fig. 1B), although the Egyptian phage is 640-nucleotides longer than phage DU_RP_I. It is unknown, however, what *Ralstonia* species or *R. solanacearum* strains the DU_RP_I phage infects. The fact that phage RsoP1EGY belongs morphologically to the family *Podoviridae* but does not share significant sequence similarity with any of the previously characterized *R. solanacearum* phages of the family *Podoviridae* suggests that the Egyptian phage maybe a new member of the family *Podoviridae*.

Fifty putative ORFs were identified in phage RsoP1EGY. Their positions, best homologs and predicted functions are summarized in the Supplementary Table S1. Among the 50 ORFs, 20 had homologs with known functions, including phage structural proteins (ORFs 38-39, 41, 46-47, and 50), early genes involved in DNA processing (ORFs 6, 18, 20,-24, 27, 30, 34, and 42), and late gene products such as lysis proteins (ORFs 2, 3, and 25). Twelve are hypothetical proteins showing marginal similarities with hypothetical proteins in *Burkholderia ubonensis* (ORF 9) or the *Ralstonia* phage DU_RP_I (ORFs 4-5, 26, 28, 31-33, 35, 40, and 48-49). Eighteen ORFs, however, had no similarity to any proteins in the databases (Table S1). The genome arrangement of phage RsoP1EGY and the 39% amino acid sequence identity between the putative RNA polymerase of phage RsoP1EGY (ORF20) and the T7 RNA polymerase (GenBank accession no. NP_041960.1) suggest that RsoP-1EGY may be a T7-like phage. A typical podovirus genome represented by *Enterobacteria* phage T7 generally consists of three functional gene clusters: cluster I for early functions, cluster II for DNA metabolism, and cluster III for structural proteins and virion assembly (Fig. 1B) [14]. We tentatively assigned the three clusters to phage RsoP1EGY and its closely related phage DU_RP_I and compared the two phages to the T7 phage (Fig. 1B). The putative cluster I of phage RsoP1EGY includes ORF8 to ORF20, containing a putative integrase (ORF18) and a RNA polymerase (ORF20) (Fig. 1B, Table S1). The putative cluster II includes ORF21 to ORF37, containing annotated phage single-stranded DNA-binding protein (ORF22), endonuclease VII (ORF24), lysozyme (ORF25), DNA primase (ORF27), DNA polymerase (ORF30), and exonuclease (ORF34) (Fig. 1B, Table S1). The putative cluster III includes ORF38 to ORF50 and ORF1 to ORF7 containing putative phage tail proteins (ORFs 38, 46 and 47), phage capsid assembly protein (ORF39), phage capsid protein (ORF41), lytic transglycosylase protein (ORF2), bacteriolytic protein (ORF3), and terminase protein large subunit (ORF6) (Fig. 1B, Table S1).

To determine the host specificity of phage RsoP1EGY, 20 *R. solanacearum* strains in our collection were tested for phage susceptibility using the method of Ahmad et al. [2]. Interestingly, none of the 10 non-r3b2 strains, but all 10

of the r3b2 strains (phylotype IIB sequevar 1) tested were susceptible to phage RsoP1EGY (Table 1). This is probably because phage RsoP1EGY was isolated from the Nile delta region of Egypt, where only r3b2 strains of *R. solanacearum* causing potato brown rot were detected [15]. Further research is needed to determine whether the Egyptian phage has potential as a biocontrol agent for r3b2 strains of *R. solanacearium* in Egypt or other countries where potato brown rot is a problem, and whether the phage can be used for specific detection of r3b2, since the phage is lytic to and specific for *R. solanacearum* r3b2.

It is interesting that phage RsoP1EGY is closely related to the *Ralstonia* phage DU_RP_I, but not to any other reported *R. solanacearum* or non-*Ralstonia* phages. It will be interesting to study the evolution of these two *Ralstonia* phages once the origin, isolation, morphology and host range of phage DU_RP_I becomes available.

Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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