Virology Reports 6 (2016) 61–73

Contents lists available at ScienceDirect

Virology Reports

journal homepage: www.elsevier.com/locate/virep

A *Ralstonia solanacearum* phage ϕRP15 is closely related to *Viunalikeviruses* and encodes 19 tRNA-related sequences

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ARTICLE INFO

Article history:
Received 1 July 2016
Received in revised form 22 July 2016
Accepted 22 July 2016
Available online 25 July 2016

Keywords:
Ralstonia solanacearum
T4-related phages
Viunalikevirus
tRNA-gene cluster

ABSTRACT

A T4-related phage infecting *Ralstonia solanacearum* was isolated in Thailand (ϕRP15). ϕRP15 virions showed unique morphology with star-like protrusions attached to the base plate via a stalk. The 168 kb genome sequence of ϕRP15 was determined and found to encode 17 tRNAs (plus two tRNA pseudogenes). Ten of these phage-encoded tRNAs corresponded to codons that are relatively abundant in the phage but rare in the host, while others matched to codons frequent in both phage and host. Phylogenetic and proteomic analyses demonstrate that ϕRP15 forms a clade with *Delftia* phage ϕW-14, which in turn is placed as a sister group of viunalikeviruses. The hosts of ϕRP15 and ϕW-14 (*R. solanacearum* and *Delftia acidovorans*, respectively) belong to Betaproteobacteria, while most of the hosts of viunalikeviruses are of the *Enterobacteriaceae* belonging to Gammaproteobacteria. These phages may have evolved closely associated with their hosts that have very different life-styles in the natural habitats.

1. Introduction

T4-like phages classically representing *Myoviridae* phages have a contractile tail sheath and infect a broad range of bacterial hosts. Studies of T4-like phage genomes as models have suggested that myovirus genomes are mosaic of conserved core genes, which include structural genes for head and tail proteins and enzyme genes for DNA and nucleotide metabolism, and the remaining variable accessory non-core genes (Filee et al., 2006). The functions of non-core genes are largely unknown, although it is assumed that they provide a selective benefit to phages (Hendrix, 2009). Petrov and coworkers recently defined “T4-related phages” to group phages that share a “Core Genome” encoding approximately 37 proteins (Lavigne et al., 2009; Petrov et al., 2010). T4-related phages include the members of the genus *T4likevirus*, the genus *SchizoT4likevirus* (http://ictvonline.org) and other phages including cyanomyoviruses and *Delftia acidovorans* phage ϕW-14. More recently, another genus “*Viunalikevirus*” inside the T4-

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http://dx.doi.org/10.1016/j.virep.2016.07.001

related phages was proposed for phages showing a number of features that distinguish them from other members of the T4-related phages (Adriaenssens et al., 2012a). Viunalikeviruses are characterized as virulent phages showing similar genome size (150–170 kbp), extensive DNA homology, strong gene synteny, and a complex adsorption apparatus on the virion. Members of this genus include phages infecting bacterial hosts belonging to *Enterobacteriaceae* (Gammaproteobacteria).

The phytopathogen *Ralstonia solanacearum*, a soil-borne Gram-negative bacterium (Betaproteobacterium), causes bacterial wilt disease in many important crops (Hayward, 1991; Yabuuchi et al., 1995). The unusually wide host-range of this bacterium extends to over 200 species belonging to >50 botanical families (Hayward, 2000). *R. solanacearum* strains constitute a heterogeneous group subdivided into five races on the basis of their host range, six biovars based on their physiological and biochemical characteristics (Hayward, 2000), and four phylotypes according to phylogenetic information (Fegan and Prior, 2005; Remenant et al., 2010). Yamada et al. (2007), Yamada (2012) and Bhunchoth et al. (2015, 2016b) isolated and characterized various types of bacteriophages infecting *R. solanacearum* strains belonging to different races and/or biovars. Among them, ϕRP15, which was initially characterized as a myovirus with a wide host-range, replicates exclusively through a lytic cycle and forms clear plaques.

2. Results

2.1. Isolation and initial characterization of ϕRP15

ϕRP15 is a myovirus isolated in Pang Nga, Thailand (Bhunchoth et al., 2016b). It formed small clear plaques with a wide range of host strains (including those isolated in Japan). In contour-clamped homogeneous electric field (CHEF) gel electrophoresis analyses, the ϕRP15 genomic DNA produced a single band of approximately 170 kbp, being nearly the same size as the T4 genome (Fig. S1). An analysis of ϕRP15 particle morphology using electron microscopy revealed characteristics of a myovirus, with an icosahedral head (diameter: approximately 90 ± 5 nm, n = 10) and a long contractile tail (length: 110 ± 10 nm, n = 10; width: 18 ± 2 nm, n = 10) (Fig. 1). The tail consists of a T4-like neck with a collar and a sheath surrounding a tail tube or core. Remarkably, ϕRP15 particles show unique star-like protrusions stemming from the baseplate via a stalk, resembling the morphology shared by viunalikeviruses, but do not show prong-like structures attached to the baseplate as observed for viunalikeviruses (Adriaenssens et al., 2012a).

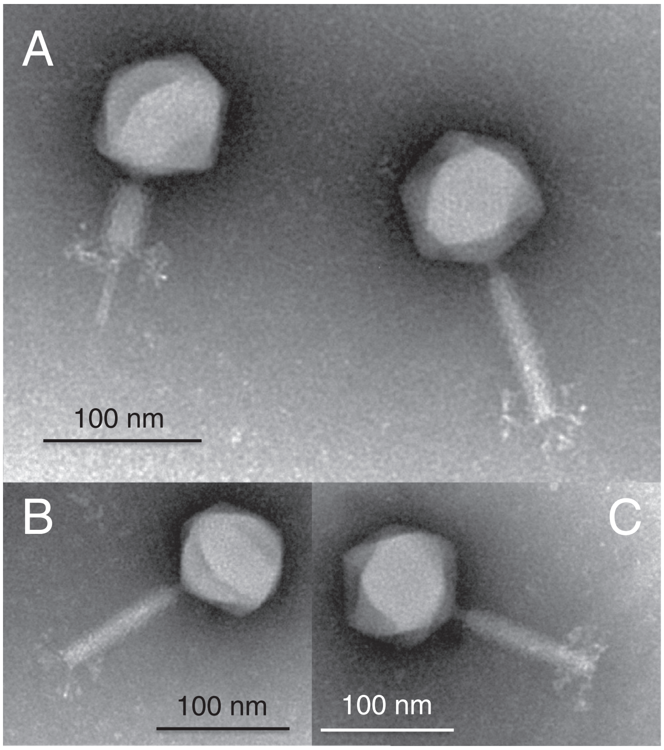

Fig. 1. Electron micrographs of ϕRP15 particles. Particles with contracted tail and uncontracted tail are compared (A). At the base plate region of uncontracted tail, umbrella-like (star-like) structures are obvious but no prong-like structures were visible (B and C). Particles were stained with 2% phosphotungstate.