

# Characterization of the Temperate Phage vB\_RleM\_PPF1 and its Site-Specific Integration into the Genome of *Rhizobium leguminosarum* Strain F1

Anupama P. Halmillawewa<sup>1,3</sup>, Marcela Restrepo-Córdoba<sup>1</sup>, Benjamin J. Perry<sup>2</sup>, Christopher K. Yost<sup>2</sup>, and Michael F. Hynes<sup>1\*</sup>

<sup>1</sup>Department of Biological Sciences, University of Calgary, 2500 University Drive NW, T2N 1N4, Canada

<sup>2</sup>Department of Biology, University of Regina, 3737 Wascana Parkway, S4S 0A2, Canada

<sup>3</sup>Department of Microbiology, University of Kelaniya, Kelaniya, Sri Lanka

## Abstract

The presence of prophages in a genome can contribute in increasing the bacterial fitness and ecological success in an environment that contains closely related phages. The temperate phage PPF1 was isolated from a lysogenized strain of *Rhizobium leguminosarum* F1. The complete genome sequence of *Myoviridae* phage PPF1 was determined using 454-Pyrosequencing technology. PPF1 is the first available complete genome sequence of a *Rhizobium leguminosarum* temperate phage (GenBank accession no: KJ746502) and the integration site and possible mechanism of integration of this phage has been identified. PPF1 is capable of efficiently lysogenizing the *R. leguminosarum* strain F1, and can be induced from its lysogenized host using UV irradiation. The genome of PPF1 is 54,506 bp in length with an average G+C content of 61.9%. The ORF predictions of the PPF1 genome revealed the presence of 94 putative proteinencoding genes and 74.5% of these predicted ORFs share homology at the protein level with previously reported sequences in the database. However, putative functions could only be assigned to 25.5% (24 ORFs) of the predicted genes. The site-specific recombination system of the phage targets an integration site that lies within a putative tRNA-Pro (CGG) gene in *R. leguminosarum* F1. Upon integration, the phage is capable of restoring the disrupted tRNA gene, owing to the 50 bp homologous sequence (*att* core region) it shares with its rhizobial host genome.

The predicted *att* site of temperate phage PPF1 share a sequence similarity with the targeted *att* site of previously characterized *Sinorhizobium meliloti* phage 16-3. In spite of phage PPF1's propensity for lysogenizing host strains, including strains on which it does not form visible plaques, there has thus far been no evidence of the presence of similar phages integrated into completed genomes of *R. leguminosarum* and related phages. The site specificity of insertion

of this phage could be used to create single copy integration vectors for genetic work in *R. leguminosarum*.

**Key words:**