proceedings

Abstract

Characterization of DNA Polymerase from *Thermus thermophilus* MAT72 Phage Tt72 †

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† Presented at Viruses 2020—Novel Concepts in Virology, Barcelona, Spain, 5–7 February 2020.

Published: 11 June 2020

Abstract: Thermophilic phages are recognized as an untapped source of thermostable enzymes relevant in biotechnology; however, their biology is poorly explored. This has led us to start a project aimed at investigating thermophilic phages isolated from geothermal areas of Iceland. In this study, we present a structural and functional analysis of the DNA polymerase of phage Tt72, which infects thermophilic bacterium *Thermus thermophilus* MAT72. An *in silico* analysis of the Tt72 phage genome revealed the presence of a 2112-bp open reading frame (ORF) encoding protein homologous to the members of the A family of DNA polymerases. It contains a conserved nucleotidyltransferase domain and a 3′ → 5′ exonuclease domain but lacks the 5′ → 3′ exonuclease domain. The amino acid sequence of Tt72 DNA polymerase shows high similarity to two as yet uncharacterized DNA polymerases of *T. thermophilus* phages: ΦYS40 (91%) and ΦTMA (90%). The gene coding for Tt72 DNA polymerase was cloned and overexpressed in *E. coli*. The Tt72 *polA* gene is composed of 2112 nucleotides. The overall G+C content of this gene is 31.58%, which is lower than the G+C content of *T. thermophilus* genomic DNA (69.49%). The Tt72 *polA* gene codes for a 703-aa protein with a predicted molecular weight of 80,477. The enzyme was overproduced in *E. coli*, purified by heat treatment, followed by HiTrap TALON column and HiTrap Heparin HP column chromatography, then biochemically characterized. The optimum activity was found at 55 °C, pH 8.5, 25 mM KCl, and 0.5 mM Mg^{2+}. Furthermore, the Tt72 DNA polymerase shows strong 3′ → 5′ exonucleolytic activity.

Keywords: *Thermus* phage; DNA polymerase; 3′ → 5′ exonuclease