Molecular cloning and characterization of Pcal_0039, an ATP-/NAD+-independent DNA ligase from hyperthermophilic archaeon Pyrobaculum calidifontis

by Qamar Abbas | Majida Atta Muhammad | Nisar Ahmad Shakir | Mehwish Aslam | Naeem Rashid | School of Biological Sciences, University of the Punjab, Quaid-e-Azam Campus, Lahore 54590, Pakistan |

DNA ligases are integral part of DNA replication, repair, and recombination. They catalyze a nick-joining reaction in double-stranded DNA. The genome sequence of the hyperthermophilic archaeon P. calidifontis contains an open reading frame, Pcal_0039, which belongs to PRK01109 superfamily and encodes a putative ATP-dependent DNA ligase comprising 583 amino acids. In this study we report on molecular cloning, heterologous production in Escherichia coli and characterization of recombinant Pcal_0039. The recombinant enzyme existed in a monomeric form in solution and displayed nick-joining activity between 40 and 85 °C. Optimal activity was observed at 70 °C and pH 5.5. Nick-joining activity was retained even after heating for 2 h at 90 °C, suggesting that Pcal_0039 is a thermostable DNA ligase. No significant shift in absorption spectra of polarized light was observed between 40 to 90 °C, when analyzed by circular dichroism spectroscopy. The nick-joining activity was metal ion dependent and Mg2+ was the most preferred. The activity was inhibited by NaCl above 100 mM and completely abolished at or above 200 mM. DNA ligases usually require ATP or NAD+ for the enzymatic activity. However, activity catalyzed by Pcal_0039 was independent of ATP or NAD+ or any other nucleotide. A mismatch adjacent to the nick, either at 3’- or 5’-end, abolished the nick-joining activity. These properties make Pcal_0039 a potential candidate for applications in DNA diagnostics. To the best of our knowledge, Pcal_0039 is the only DNA ligase, characterized from genus Pyrobaculum, which exhibits optimum nick-joining activity at pH below 6.0 and Cover Letter independent of any nucleotide cofactor. High thermostability and discrimination of mismatched nucleotides at the nick-point makes Pcal_0039 a potential candidate for detection of single nucleotide substitution.

Keywords: DNA ligase; Heterologous expression; Hyperthermophilic archaeon; Nick-joining; Pyrobaculum calidifonti