The quest of thermophilic microorganisms capable of producing robust esterase enzymes with the resilience to high temperatures has opened new prospects for the biotechnology industry. The peptidase known as S9 prolyl oligopeptidase catalytic domain protein (S9) sourced from *Thermotoga maritima*, boasting a sequence of 921 bp encrypting 306 a.a was cloned into a mesophilic host generating a thermotolerant recombinant esterase. Initially, S9 was cloned in the pTZ57R/T cloning vector, sub-cloned into the exp. vector pET-21a(+) and successively overexpressed in *Escherichia coli* BL21 CodonPlus (DE3)-RIPL. Through multiple sequence alignment, it was revealed that the esterase (S9) exhibited a remarkable 100% homology with the S9 peptidase of *T. maritima*. Furthermore, an in-depth analysis of the protein's 3D structure confirmed the pivotal catalytic role of Ser^{160}, His^{246}, and Asp^{196} within the catalytic triad. For induction, IPTG was employed, leading to the successful heterologous expression of the protein. This accomplishment was confirmed through SDS-PAGE analysis, which shown MW of S9 was 35 kilo Daltons. Moreover, cell fractionation of the recombinant S9 coupled with preliminary characterization showed the enzyme's peak activity (360 U/mL/min) with pNP-acetate as the substrate, operating optimally at 80 ° temperature and pH 6.0 within the intracellular cell lysate fraction. Conclusively, this study represents a significant progress in showcasing the potential of the recombinant esterase (S9) for application in biodiesel production and a range of other high-temperature biotechnological processes.