

CLONING OF DNA POLYMERASE-1 GENE FROM THERMOPHILIC *Bacillus licheniformis* STRAIN NWMF1 INTO AN *E.coli* EXPRESSION SYSTEM.

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Abstract

DNA polymerase, catalyze template directed synthesis of DNA from nucleotide triphosphate. Thermostable DNA polymerase-| (DNAP-1) has been a common reagent in molecular biology because of its use in DNA amplification and DNA sequencing by PCR. DNAP-1 produced in moderate thermophiles such as *Bacillus* species may not be suitable for PCR, However, moderately thermophilic DNAP-1 from *Bacillus* has been used in molecular biology techniques such as loop mediated isothermal amplification. It is a low cost alternative to detect certain infectious diseases such as tuberculosis, malaria and can be applied in low/middle income countries. The objective of the study was isolation and cloning of DNAP-1 gene from native thermophilic bacterium, *Bacillus licheniformis* strain NWMF1 and over-expression by using expression host *E. coli* BL21(DE3)pLysS. A gram ve endospore forming thermophilic bacterium was isolated from soil near the hot-water springs at Polonnaruwa, Sri Lanka. The identification of *Bacillus licheniformis* strain NWMF1 was carried out using morphological tests and 16s r.RNA gene sequence analysis. Initially the gene was cloned into pGEMT-easy vector and transformed into *E. coli* JM109 followed by sequence confirmation and protein blast analysis by NCBI. Thereafter the DNAP-1 gene re-cloned into PET28a vector and transformed into *E. coli* BL21(DE3)pLysS expression host. Recombinant *E. coli* clones were confirmed by colony PCR. Sequence analysis confirmed the presence of the complete gene (2640bp) including start and stop codons. The complete protein sequence consists 879 amino acids. SDS-PAGE and analysis by EXPASy–ProtParam indicated the molecular weight of DNAP-1 as ~92 kDa. Polymerase activity of His-tag purified DNAP-1 was demonstrated by PCR methodology.

Subjects

[SRI Lanka; MOLECULAR cloning; BACILLUS licheniformis; MOLECULAR biology; GENE amplification; THERMOPHILIC bacteria; MIDDLE-income countries](#)

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