ABSTRACT

This study is based on the cloning and sequencing of a gene (Pcal_0976) encoding thermostable pullulanase from the hyperthermophilic archaeon *Pyrobaculum calidifontis*. The full length gene consists of 1140 nucleotides and codes for a protein of 379 amino acids including 28 amino acids residues of predicted signal peptide. The predicted mass of protein before processing of signal peptide was 40.5kDa while after signal peptide cleavage the mass of protein was predicted to be 37.8 kDa. DNA fragment corresponding to mature pullulanase (1059 bp) was PCR amplified and cloned in cloning vector pTZ57R. Cloning of gene was confirmed by determining the DNA sequence. The GC content of the gene was 56.84 % that was similar to the value reported for the whole genome of *P. calidifontis*. The gene was then cloned in expression vector pET-21a (+). Heterologous expression of the gene could not be achieved in *Escherichia coli* even with induction at 1.0 mM IPTG concentration. Amino acid sequence of Pcal_0976 displayed highest homology (76-81%) with other pullulanases from Thermoproteaceae and upto 34% homology with the pullulanases from *Desulfurococcaceae* and *Thermococaceae*. The four regions conserved among amylolytic enzymes could not be identified in the sequence of pullulanase. Codon usage of the gene was similar to that found for other genes in the genome of *P. calidifontis*.