

**Cold-adapted RTX lipase from antarctic *Pseudomonas* sp. strain AMS8 : isolation, molecular modeling and heterologous expression.**

ABSTRACT

A new strain of psychrophilic bacteria (designated strain AMS8) from Antarctic soil was screened for extracellular lipolytic activity and further analyzed using molecular approach. Analysis of 16S rDNA showed that strain AMS8 was similar to *Pseudomonas* sp. A lipase gene named lipAMS8 was successfully isolated from strain AMS8, cloned, sequenced and overexpressed in *Escherichia coli*. Sequence analysis revealed that lipAMS8 consist of 1,431 bp nucleotides that encoded a polypeptide consisting of 476 amino acids. It lacked an N-terminal signal peptide and contained a glycine- and aspartate-rich nonapeptide sequence at the C-terminus, which are known to be the characteristics of repeats-in-toxin bacterial lipases. Furthermore, the substrate binding site of lipAMS8 was identified as S207, D 255 and H313, based on homology modeling and multiple sequence alignment. Crude lipase exhibited maximum activity at 20 C and retained almost 50 % of its activity at 10 C. The molecular weight of lipAMS8 was estimated to be 50 kDa via sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The optimal expression level was attained using the recombinant plasmid pET32b/BL21(DE3) expressed at 15 C for 8 h, induced by 0.1 mM isopropyl  $\beta$ -D thiogalactoside (IPTG) at *E. coli* growth optimal density of 0.5.

**Keyword:** Cold-adapted; lipAMS8; RTX repeats; Signal peptide.