

Gene Cloning and Enzyme Characterization of L-aminoacylase from *Deinococcus radiodurans* CCRC12827

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ABSTRACT

Two genes (DR1711 and DR0339) on *Deinococcus radiodurans* R1 genome had high similarity with L-aminoacylase (laa) genes from different species. So both genes were used as template to design primer pairs. Chromosome DNA of the radioactivity resistant bacterium *D. radiodurans* CCRC 12827 was used as template to amplify laa1 and laa2 genes by PCR, respectively. Cloning of laa1 or laa2 genes into *Escherichia coli* and expression of L-aminoacylase activity were performed. The length of laa1 open reading frame (ORF) was 1,167 bp, however, the laa2 ORF was 1,179 bp. The molecular weight of the translation product LAA1 and LAA2 were 41,443 Da and 42,607 Da, respectively. Both LAA1 and LAA2 were intracellular enzymes. Sequence alignment between LAA1 and *D. radiodurans* R1 N-acyl-L-amino acid amidohydrolase shows 98% identity, while comparison of LAA2 and *D. radiodurans* R1 probable N-acyl-L-amino acid amidohydrolase shows 99% identity. The *D. radiodurans* L-aminoacylase gene was cloned into pQE30 expression vector and transformed to *E. coli* Novus Blue. Finally, we used Co-NTA column to purify the enzymes. The optimal temperatures of enzymes were measured as 45 °C and 35 °C for LAA1 and LAA2, respectively. The optimal pH for LAA1 and LAA2 were pH 8.0 and pH 7.0, respectively. Without metal ion, LAA1 and LAA2 were inactivated. Mn²⁺ and Co²⁺ ions promote enzyme activity, because both enzymes could be metalloenzymes. Besides, using N-CBZ-Gly-Ala as substrate, LAA1 and LAA2 showed maximal carboxypeptidase specific activity. When the substrate switched to N-acetyl-L-His, LAA1 showed the best L-aminoacylase activity. Using N-chloroacetyl-L-Phe as substrate, LAA2 had maximal L-aminoacylase specific activity. In addition, LAA2 had dipeptidase activity of hydrolyzing the dipeptide L-ornithine-L-alanine. These results indicate that LAA1 is a bifunctional enzyme and LAA2 is a trifunctional enzyme.

Keywords : *Deinococcus radiodurans*, L-aminoacylase, gene cloning, enzyme activity assay.

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