A NEWLY IDENTIFIED GLUTAMINASE-FREE L-ASPARAGINASE (L-ASPG86) FROM THE MARINE BACTERIUM *Mesoflavibacter zeaxanthinifaciens*

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L-Asparaginase (EC 3.5.1.1) is an enzyme involved in asparagine hydrolysis and has the potential to effect leukemic cells and various other cancer cells. We identified the L-asparaginase gene (L-ASPG86) in the genus *Mesoflavibacter*, which consists of a 1035-bp open reading frame (ORF) encoding 344 amino acids (aa). Following phylogenetic analysis, the deduced amino acid sequence of L-ASPG86 (L-ASPG86) grouped as a type I asparaginase with respective homologs in *Escherichia coli* and *Yersinia pseudotuberculosis*. The L-ASPG86 gene was cloned into the pET-16b vector to express the respective protein in *E. coli* BL21 (DE3) cells. Recombinant L-asparaginase (r-L-ASPG86) showed optimum conditions at 37-40°C, pH 9. Moreover, r-L-ASPG86 did not exhibit glutaminase activity. In the metal ions test, its enzymatic activity was highly improved upon addition of 5 mM manganese (3.97-fold) and magnesium (3.35-fold) compared with the untreated control. The specific activity of r-L-ASPG86 was 687.1 units/mg under optimum conditions (37°C, pH 9 and 5 mM $MnSO_4$).

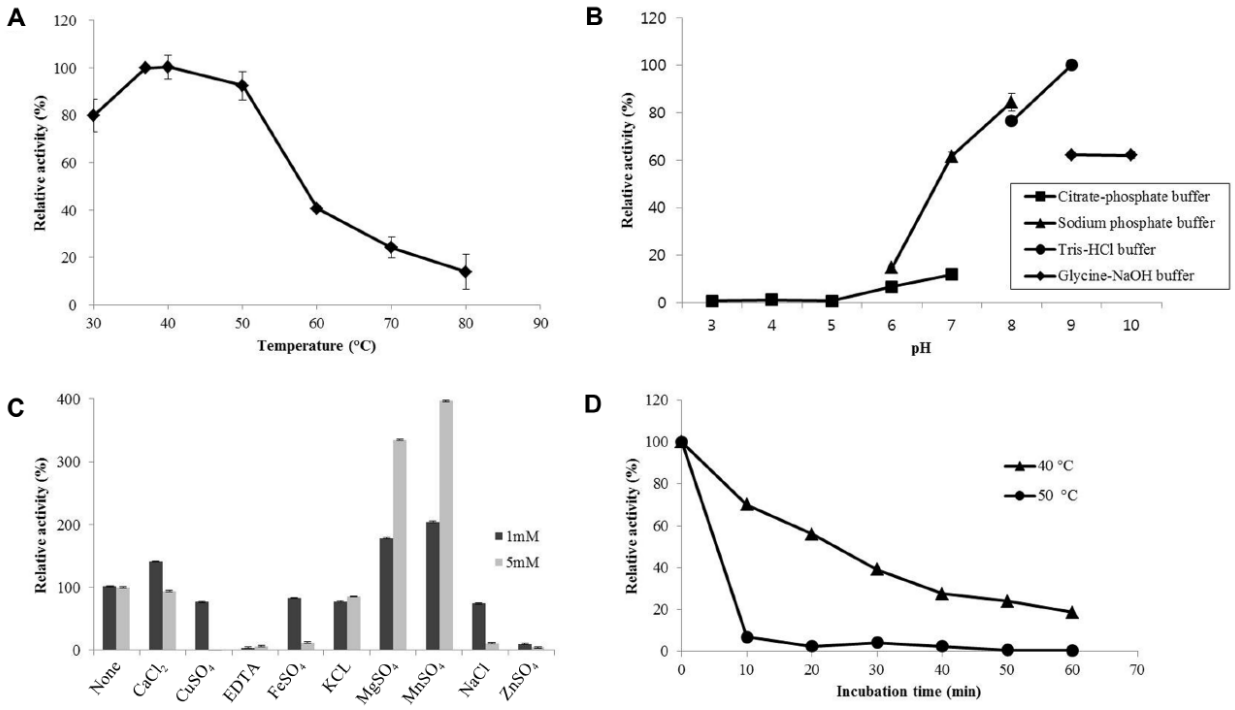

Figure 1. Biochemical properties of purified r-L-ASPG86. (a) Effects of temperature on r-L-ASPG86 activity at temperature in the range of 30-80 °C. (b) Effects of pH on r-L-ASPG86 activity at pH ranging from 3-10. (c) Effects of metal ions and chelating agents on r-L-ASPG86 activity with 1 mM and 5 mM, measured after incubation at 37 °C for 10 min. (d) Effects of thermostability on remaining r-L-ASPG86 activity at 40 °C and 50 °C for 0-60 min.