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Cloning, expression, and characterization of a novel marine L-asparaginase from *Pseudomonas aeruginosa HR03*

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Abstract

The present study focused on the cloning, expression, and characterization of marine L-asparaginase of *Pseudomonas aeruginosa HR03* isolated from fish intestine (*L.klunzingeri*). Marine *Pseudomonas aeruginosa HR03* was used for retrieving the l-asparaginase encoding gene (HR03Asnase) of size 936 bp. The gene was successfully cloned into the pET21a vector and expressed into *Escherichia coli BL21* (DE3) for characterization of the protein. The recombinant HR03Asnase enzyme was purified by affinity chromatography using nickel affinity chromatography, and the enzymatic properties of HR03Asnase, including the effects of pH and temperature on HR03Asnase activity and kinetic parameters, were determined.

The recombinant enzyme HR03Asnase showed the highest similarity to type I bacterial L-asparaginase from *Pseudomonas aeruginosa*. The three-dimensional (3D) modeling results indicate that HR03Asnase exists as a homotetramer. Also, The Molecular weight analysis using SDS-PAGE revealed ~35 kDa. The HR03Asnase showed optimum pH and temperature of 8.0 and 40 °C, respectively. The maximum activity of HR03Asnase was reduced by 50% at 90 °C after 10-min incubation; though, the enzyme preserved more than 20% of its activity after 30-min incubation. This enzyme also preserved almost 50% of its activity at pH 12 after 40-min incubation. The km and Vmax of the enzyme obtained with l-asparagine as substrate were 10.904 mM and 3.44 × 10−2 mM/min, respectively.

The recombinant HR03Asnase of marine *P. aeruginosa* may also be explored as a potential agent in pharmaceutical and food applications. The assessment of pH and temperature stability of HR03Asnase showed that the enzyme has a wide range of activity, which is a suitable characteristic for

its application in different industries. Overall, the results of the present study show that marine sources are promising biological reservoirs for enzymes to be used for biotechnological purposes, and marine thermostable HR03Asnase is likely a potential candidate for its future usage in the pharmaceutical and food industries.

Key words:
Purification , Cloning, E. coli, Enzyme activity, Acrylamide

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