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Subcloning and expression of recombinant glucose-dehydrogenase from *Bacillus subtilis*

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Abstract

Glucose-dehydrogenase (GDH) is an enzyme that catalyzes the oxidation of glucose to gluconate, reducing NAD(P)+ to NAD(P)H + H+. This enzyme is of particular interest due to the fact that it can be used to regenerate the NAD cofactor using a cheap substrate such as glucose. The aim of this study was the fusion of recombinant GDH gene with the His-Tag at the N-terminus. For this purpose, the recombinant GDH gene was subcloned into the pET28a expression vector and expressed in *E. coli* BL21(DE3) cells. The recombinant protein was expressed both in soluble form (10% of total proteins) and in inclusion bodies. The recombinant GDH was purified by Ni-agarose affinity chromatography and tested for enzymatic activity (glucose and NAD). In conclusion, after subcloning the ORF did not change, the protein was fused with His-tag, and this fusion did not affect the activity or solubility of the enzyme. The results obtained in this study may be used to optimize glucose-dehydrogenase production so that it would increase its biotechnological importance.

Keywords: cofactor regeneration; glucose-dehydrogenase; recombination; subcloning; vector

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