Cloning and Heterologous Expression of Glucose Oxidase Gene from Aspergillus niger Z-25 in Pichia pastoris

A gene of glucose oxidase (GOD) from Aspergillus niger Z-25 was cloned and sequenced. The entire open reading frame (ORF) consisted of 1,818 bp and encoded a putative peptide of 605 amino acids. The gene was fused to the pPICZαA plasmid and overexpressed in Pichia pastoris SMD1168. The recombinant GOD (rGOD) was secreted into the culture using MF-α factor signal peptide under the control of the AOX1 promoter. Sodium dodecyl sulfate polyacrylamide gel electrophoresis indicated that rGOD exhibited a single band at around 94 kDa. The maximal GOD activity of approximately 40 U/mL was achieved in shake flask by induction under optimal conditions after 7 days. rGOD was purified by ammonium sulfate precipitate leading to a final specific activity of 153.46 U/mg. The optimum temperature and pH of the purified enzyme were 40 °C and 6.0, respectively. Over 88% of maximum activity was maintained below 40 °C. And the recombinant enzyme displayed a favorable stability in the pH range from 4.0 to 8.0. The Lineweaver–Burk plotting revealed that rGOD exhibited a Km value of 16.95 mM and a Kcat value of 484.26 s⁻¹.

Keyword: Glucose oxidase, Overexpression, Pichia pastoris, Aspergillus niger, Properties