Cloning, extracellular expression and characterization of predominant β-CGTase from Bacillus sp. G1 in E. coli.

ABSTRACT

The cyclodextrin glucanotransferase (CGTase, EC 2.4.1.19) gene from Bacillus sp. G1 was successfully isolated and cloned into Escherichia coli. Analysis of the nucleotide sequence revealed the presence of an open reading frame of 2,109 bp and encoded a 674 amino acid protein. Purified CGTase exhibited a molecular weight of 75 kDa and had optimum activity at pH 6 and 60 degrees C. Heterologous recombinant protein expression in E. coli is commonly problematic causing intracellular localization and formation of inactive inclusion bodies. This paper shows that the majority of CGTase was secreted into the medium due to the signal peptide of Bacillus sp. G1 that also works well in E. coli, leading to easier purification steps. When reacted with starch, CGTase G1 produced 90% β-cyclodextrin (CD) and 10% γ-CD. This enzyme also preferred the economical tapioca starch as a substrate, based on kinetics studies. Therefore, CGTase G1 could potentially serve as an industrial enzyme for the production of β-CD.

Keyword: Bacillus sp. G1; Cyclodextrin; Cyclodextrin glucanotransferase; Extracellular expression; Predominant β-CGTase; Signal peptide.