Molecular expression of a recombinant thermostable bacterial amylase from geobacillus stearothermophilus SR74 using methanol-free meyerozyma guilliermondii strain SO yeast system

ABSTRACT

α-Amylase, which was isolated from Geobacillus stearothermophilus SR74, has shown its potential to be used in industrial applications. However, its expression in the Pichia pastoris expression system with the alcohol oxidase 1 promoter (PAOX1) requires high methanol consumption and is time-consuming. This study aimed to express SR74 α-amylase in an alternative yeast system, using Meyerozyma guilliermondii strain SO, which was isolated from a spoiled orange (SO) under the regulation of a formaldehyde dehydrogenase promoter (PFLD). Qualitative screening showed that strain SO possessed a native amylase grown on YPD-starch plate at 30 °C. The recombinant SR74 α-amylase was further quantified and validated using the Western blot test. It was confirmed that SR74 α-amylase was expressed by strain SO extracellularly with a size of 59 kDa. Optimization in a shake flask showed that the recombinant SR74 α-amylase, which was regulated by PFLD, was successfully produced (26 U/mL) without any external inducer in the YPT medium after 24 h of cultivation. In conclusion, strain SO was able to produce SR74 amylase without methanol in one-fifth the fermentation time of P. pastoris. Further optimization of the expression may be done to improve the yield, as this methanol-free host is still underexplored.

Keyword: SR74 α-amylase; FLD promoter; Meyerozyma guilliermondii strain SO; Yeast; Pichia sp.; Amylase; Geobacillus stearothermophilus