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A new thermostable β-glucosidase mined from *Dictyoglomus thermophilum*: Properties and performance in octyl glucoside synthesis at high temperatures

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ABSTRACT

A new β-glucosidase (DtGH) representing 40% identity with an apple seed glycosidase (ASG) was cloned from *Dictyoglomus thermophilum*. DtGH showed extremely high thermostability in aqueous solution, with half-lives of 533, 44, and 5 h measured at 70, 80 and 90 °C, respectively. Therefore it was used for direct glycosylation of *n*-octanol at 70 °C instead of 50 °C as usually. As a result, the glucose based conversion was increased by 27%, but the time spent to reach equilibrium was decreased from 7 d to 3 d. This enzyme also exhibited excellent stability under the reaction environment, retaining 70–80% of its initial activity after 7 d of incubation at 70 °C in either 1.7 M glucose solution or octanol-aqueous (85:15, v/v) system. It could retain part of synthetic activity even in boiling water. Owing to the strong glucose-tolerance and extremely high thermostability, DtGH should be promising for various glucosides synthesis.

1. Introduction

Alkyl β-D-glucosides, a group of green and non-ionic surfactants with good emulsifying function and antimicrobial activities, are widely used in pharmaceuticals, detergents, and food ingredients (Bhatia et al., 2002; Sarney and Vulfson, 1995). The enzymatic synthesis of alkyl glucosides has many attractive features: the reaction can be performed under mild conditions; it can avoid tedious protection/deprotection steps and meet the requirements of environmental safety and cleanliness (Lu et al., 2007; Vic et al., 1997). Enzymes with transglycosylation activities are thus of steadily increasing interests (Pal et al., 2010). Almond β-glucosidase has been widely used as a commercial enzyme in the condensation reaction between glucose and alcohols (Ducret et al., 2002, 2006; Tong et al., 2005). However, the time spent to reach equilibrium of the reversed hydrolysis reaction was very long even if the reaction temperature was set at 50 °C. In this study, an optional strategy of using a more thermostable β-glucosidase to catalyze reversed hydrolysis reaction might partially solve this problem. The reaction kinetics could be relatively more advantageous at an elevated temperature, especially when longer-chain alcohols are used as substrates. Moreover, the low solubility of the reactant could also be significantly compensated (de Roode et al., 2001).

Although plenty of thermostable β-glucosidases have been identified and characterized in recent years, few of them were used in the synthesis of alkyl glucosides through the reverse hydrolysis method. The main bottleneck is that β-glucosidases are always sensitive to high glucose concentration (Ducret et al., 2002; Wallecha and Mishra, 2003). Moreover, in the water-poor reaction system, the deactivation of enzyme may be fast due to the presence of organic solvents and the Maillard reactions at high temperatures (de Roode et al., 2001; Fischer et al., 1996). Even a very stable β-glucosidase from *Pyrococcus furiosus* was rapidly deactivated in the direct glucosylation reaction system before the thermodynamic equilibrium was reached (de Roode et al., 2001).

It is found that β-glucosidases from plant seeds are often highly glucose-tolerant since a large amount of glucose is liberated during germination. In our previous research, a β-glycosidase from apple seed (ASG) was characterized as a new promising source for alkyl *O*-glucoside synthesis by reverse hydrolysis (Tong et al., 2004; Yu et al., 2007). Compared to the commercial almond β-glucosidase (Ducret et al., 2002, 2006; Tong et al., 2005), it is abundant, stable, and cheap. With the aim of exploring novel β-glucosidases that can realize the condensation reaction at even higher temperatures, an extensive mining *in silico* for glucosidases from thermophiles was performed using the gene of ASG as the probe. As a result, a thermostable β-glucosidase (DtGH) with a sequence similar to ASG was

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