Key Engineering Materials
ISSN: 1662-9795, Vol. 874, pp 96-106

Submitted: 2020-07-01
Revised: 2020-08-04
Accepted: 2020-08-05
Online: 2021-01-22

Recombinant Expression and Biochemical Characterization of Levansucrase from Halophilic Bacteria *Bacillus licheniformis* BK1 and BK2

Nur Umriani Permatasari[1,a], Enny Ratnaningsih[2,b] and Rukman Hertadi[2,c*]

[1]Chemistry Department, Faculty of Mathematics and Natural Sciences, Hasanuddin University, Indonesia

[2]Biochemistry Research Division, Faculty of Mathematics and Natural Sciences, Institut Teknologi Bandung, Indonesia

[a]nurumriani@unhas.ac.id, [b]enny@chem.itb.ac.id, [c*]rukman@chem.itb.ac.id

Keywords: levansucrase, *E. coli* BL21 (DE3)pLysS, full factorial CCD, exopolysccaharides, moderately halophilic bacteria.

Abstract. Levansucrase was an extracellular polysacharride (EPS) which has a role in synthesizing levans by transferring fructose moiety from sucrose to acceptor molecules. In the previous study, we have successfully cloned the levansucarese gene from two *Bacillus licheniformis* strains of BK1 and BK2 labeled as *lsbl-bk1* and *lsbl-bk2*. The present study aims to optimize the expression level of both genes in *E. coli* expression system and also to obtain the optimum conditions for the recombinant enzymes activity by applying the response surface methodology (RSM). The optimization result found that the highest Lsbl-bk1 production in *E. coli* expression system was occurred when the recombinant cells grown in the medium containing 0.6% (w/v) NaCl at 42ºC, and induced by 0.6 mM IPTG. Different optimum conditions were found for Lsbl-bk2 production. It was achieved when 1.1% (w/v) NaCl added to the production medium and induced by 0.7 mM IPTG at 40ºC. RSM optimization result for biochemical characterization of Lsbl-bk1 levansucrase showed the highest specific activity achieved at 56ºC and pH 7.5, whereas for the Lsbl-bk2 levansucrase reached the highest specific activity at 50ºC and pH 7.5. The addition of Co^{2+}, Ti^{2+}, Mg^{2+}, Ba^{2+}, Zn^{2+}, Fe^{3+}, Ca^{2+} metal ion to both levansucrases solution did not significantly altered their specific activity, indicating that both levansucrases are not metalo enzymes. Furthermore, the specific activity of levansucrase was also not affected by the addition of 1-25% (w/v) NaCl, suggesting that the variation of ionic strength did not alter the native state of both enzymes. The plot results of levansucrase specific activities toward sucrose concentration showed that both levansucrases follow Michaelis-Menten profile with k_{cat}/K_M values about 3.8 and 3.6 s^{-1}/mM respectively. These data indicated that the recombinant levansucrases from halophilic bacteria *B. licheniformis* BK1 and BK2 are a non metaloenzyme with high affinity and binding rate to sucrose substrate, in which the catalytic efficiency on hydrolysis reactions is relatively low.