

ABSTRACT

The present study describes the effect of lactose and selection of medium for the expression of recombinant α-amylase from Thermotoga sp. in E. coli BL21 codon plus. The α-amylase was cloned previously in PET28a expression vector under the influence of T7lac promoter. Recombinant E. coli strain was grown in five different media (3xZYB, 3ZYBM9, ZYBM9, 4xZB & LB). Difference in expression of recombinant α-amylase on induction with IPTG and lactose in each media was determined in term of enzyme activity, protein contents and specific activity. Among all the media, 4xZB and ZYBM9 gave 13.13±0.84 and 13.73±2.01 units with 100 mM lactose 48 hrs after induction. Due to statistically insignificant difference in α-amylase expression in the two media, both were tested for the effect of different concentrations of lactose as inducer. Both the extra- and intracellular expression of α-amylase by host cells was comparatively higher in 4xZB medium on induction with 200 mM lactose. The enzyme expression was high when E. coli BL21 Codon Plus cells, grown to 0.6 OD_{600} , were kept at 42°C for I hr (200 rpm) immediately after induction. The maximum production of the recombinant α-amylase (extracellular 20.3±1.163 U/ml/min and intracellular 41.99±1.64 U/ml/min) and protein (extracellular 0.505 mg/ml and intracellular 6.8 mg/ml, respectively) were obtained when E. coli culture was incubated at 37°C for 48 hrs after induction with 200 mM lactose.