



ABSTRACT

The present study deals with the high-titre biotransformation of xylose to xylitol by using a mutant strain of *Bacillus subtilis*. Bacterial strain was improved by chemical method as well as different cultural parameters were optimized. Solid state fermentation was carried out using sugarcane bagasse as a substrate. The substrate was pre-treated by soaking in different concentration of liq. NH_3 (0, 0.5, 1, 1.5, 2, 2.5 and 3%) for different time periods (1-6 min). From treated bagasse (2% for 5 min) 7.50 ± 0.37 mg/g xylitol was produced. Strain improvement was done by induced chemical mutagenesis of wild-type *B. subtilis* IIB-09 using MMS. Different MMS concentrations (0.5, 1, 1.5, 2, 2.5 and 3 mM) and different exposure time (5-30 min) were investigated. The final mutant derivative (MMS-Fc-C5) was able to produce 11.90 ± 0.6 mg/g of xylitol which is highly significant (*HS*, $p \leq 0.05$). The selected wild and mutant strains were optimized for various parameters viz. substrate level (3.12 g), moistening medium volume (5 ml), incubation time (72 h) and inoculum size (20%). Scanning electron microscopy revealed morphological characteristics of wild-type and mutant strain. Further, effect of various additives (microminerals, stimulators and inhibitors) on xylose reductase (XR) activity was evaluated. The most notable finding was with the addition of microminerals (CaCO_3 15 mM, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 10 mM) which enhanced the enzyme activity significantly. However, addition of EDTA and SDS declined the activity of XR. The final mutant strain produced 485 ± 4.85 U/g of XR which is 1.15-fold higher than the wild-type. The enzyme was partially purified with 70% ammonium sulphate precipitation and followed by dialysis. The yield of mutant strain after purification was found to be 76%. The molecular weight of the enzyme from mutant strain was found to be 33 kDa. It was concluded that the mutant strain exhibited higher xylitol and XR production, thus could be commercially an attraction for food and pharmaceutical industries.