

Enhanced cellulase production by a novel thermophilic *Bacillus licheniformis* 2D55: characterization and application in lignocellulosic saccharification

ABSTRACT

Effects of nutritional and physicochemical factors were investigated for cellulase production by the newly isolated thermophilic strain *Bacillus licheniformis* 2D55 (Accession No. KT799651). The optimum cellulase production in shake flask fermentation was attained at 60 °C, pH 3.5, 180 rpm, and in a medium containing untreated sugarcane bagasse and pre-treated rice husk at 7% (w/v), urea, 1 g/L, peptone, 11.0 g/L, Mg(SO₄)₂, 0.40 g/L, CaCl₂, 0.03 g/L, Tween 80, 0.2% (w/v), and 3% inoculum. The highest carboxymethyl cellulase (CMCase), filter paperase (FPase), and β-glucosidase produced under the optimized conditions were 29.4 U/mL, 12.9 U/mL, and 0.06 U/mL, respectively, after 18 h of fermentation. Optimization of the parameters increased the CMCase, FPase, and β-glucosidase activities by 77.4-fold, 44.5-fold, and 10-fold, respectively. The crude enzyme was highly active and stable over broad temperature (50 to 80 °C) and pH (3.5 to 10.0) ranges with optimum temperature at 65 °C and 80 °C for CMCase and FPase, respectively. The optimum pH for CMCase and FPase was 7.5 and 6.0, respectively. Saccharification of sugarcane bagasse and rice husk by crude cellulase resulted in perspective yields of 0.348 and 0.301 g g⁻¹ dry substrate of reducing sugars. These results suggest prospects of thermostable cellulase from *B. licheniformis* 2D55 in application for bio-sugar production and other industrial bioprocess applications involving high temperatures.

Keyword: *Bacillus licheniformis* 2D55; Thermostable cellulase; Lignocellulosic saccharification; Optimization