Hydrolysis of banana wastes by lignocelluloses degrading enzymes from newly isolated thermophilic *Bacillus* sp. AAX01

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Recently, researchers have focused on alternative energy sources for some reasons such as the reduction of fossil fuel reserves, the increase of energy consumption parallel to the population increase, the development of the industrial economy and environmental pollution. Lignocellulosic biomass is one of the important alternative energy sources because it is inexpensive and renewable. Biofuels can be produced by fermentation of sugars obtained as a result of hydrolysis of lignocellulosic biomass, which usually contains 40-50 % cellulose, 25-35 % hemicelluloses and 15-20 % lignin (Udeh, 2017). The use of lignocellulose-based biofuels provides opportunities for reduction of the greenhouse gas emissions and the global warming.

Banana, which is largely produced in India, China, Uganda, Ecuador and Brazil, is one of the world's most important and preferred fruit. In Turkey, they are produced in Mersin and Antalya province (especially Anamur, Bozyazı, Alanya and Gazipasa districts) which are located to the Mediterranean region of country. The annual bananas productions in these cities are approximately 206 thousand tons (Subaşı, 2016). Taking into account that approximately 3 tons of pseudostem, 0.16 tons of stems and 0.48 tons of leaves were generated for every ton of biomass harvested, banana wastes, which are generally burned, can be used as substrate for microbial enzyme and converted into sugar syrup by hydrolysis.

In this study, thermopilic bacterial strains, which produce cellulases were isolated from soils contaminated with banana wastes in Mersin. The lignocellulose degrading enzymes were produced by selected isolate using banana leaves as primary carbon sources. Additionally, the hydrolytic potentials of enzymes against banana wastes such as banana leaves and banana pseudostem were evaluated.

One gram of soil was added into 100 mL Minimal Salt Medium (MSM) supplemented with 10 g/L carboxymethyl cellulose (CMC) and incubated at 50 °C overnight. At the end of the incubation, serial dilutions (10^{-3}-10^{-6}) were spread on the CMC agar plates (10 g/L CMC, 1 g/L KNO_3, 0.5 g/L NaCl, 0.5 g/L K_2HPO_4, 0.5 g/L $MgSO_4$. $7H_2O$, 0.5 g/L $FeSO_4$. $7H_2O$ and 20 g/L agar) and incubated at 50 °C for 2 days. Morphologically different colonies were inoculated on CMC agar plates by sterile tooth pick to compare its cellulase production titre. After incubation at 50 °C for 2 days, plates were flooded with 0.1 % Congo red solution and 0.1 % NaCl for 15 min, respectively. The ratio of the diameter of clear zone formed around the colony and the diameter of colony was calculated. Isolate AAXO1 was selected for further enzyme production, because it showed maximum cellulase production on agar plates. The 16S rDNA was amplified using 27F' (50-AGAGTTTGATCCTGGCTCAG-30) and 1494R' (50-CTACGGCTACCTTGTTACGA-30) primers. Then, PCR products were sequenced. 16S rDNA sequences of isolate AAXO1 were compared with sequences in NCBI using BLAST algorithm. Isolate AAX01 was identified as members of Bacillus. Additionally, it was found that similarity between 16S rDNA sequences of isolate AAXO1 and *Bacillus subtilis* AI-2 (NCBI Accession Number: KU681044.1) was 99 %. Phlogenetic position of *Bacillus* sp. AAX01 was observed in Figure 1.

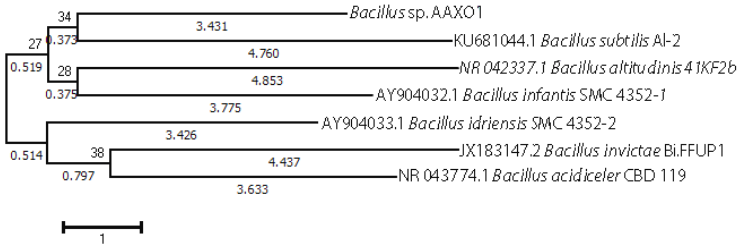

Figure 1. Phylogenetic position of *Bacillus* sp. AAXO1. Phylogenetic tree was drawn by Mega 6.

The effects of incubation time (0-7 days), temperature (30-60 °C), initial pH of medium (pH 5-10) and agitation rate (0-200 rpm) on cellulase production by *Bacillus* sp. AAX01 in MSM medium supplemented with 10 g/L banana leaves were determined. *Bacillus* sp. AAX01 produced approximately 4.9 U/ml CMCase, 1.3 U/mL FPase and 1.7 U/mL avicelase in optimum condition. While Peroxidise and xylanase activities were 0.3 U/mL and 3.9 U/Ml in culture supernatant, laccase, β-glucosidase, β-xylosidase and α-L arabinofranosidase activities were not detected. Additionally, total protein was estimated as 0.2 mg/mL. Enzyme cocktail was

concentrated by 10 kDa cut of ultrafiltration membrane. Stabilities of crude enzymes at 50-70 °C were determined.

The hydrolysis experiments performed in 150 mL Erlenmeyer flasks containing 50 mL mixture of 50 mM phosphate buffer (pH 7.0), 2.5 g banana waste (banana leaves and banana pseudostem), which had different particle sizes (0.25, 0.5 and 1 mm) and 2.5 mg enzyme cocktail. The flasks were incubated at 50 °C and 150 rpm for 48 h. Aliquots were drawn in regular intervals and then reducing sugar and total phenol concentrations in samples were determined by DNS (Adıgüzel, 2016) and Folin-Ciocalteau methods (Tuncer, 2002), respectively. The reducing sugar concentration reached 197.9, 117.5 and 95.86 mg/g biomass after 24h hydrolysis of banana leaves, which have 0.25, 0.5 and 1 mm particle sizes, respectively (Figure 2a). It was determined that 218.9, 133.52 and 109.98 mg/g biomass reducing sugars were obtained by 24 h hydrolysis of 0.25, 0.5 and 1 mm banana pseudostems, respectively (Figure 2b). Additionally, it was observed that prolonged hydrolysis time beyond 24 h, had no significant effect on the increasing reducing sugar. After hydrolysis of banana leaves and banana pseudostems, total phenol concentrations were 40-47 and 35-45mg/g biomass in hydrolysates, respectively (Figure 3). No significant difference was observed in the total amount of phenol released after the hydrolysis of banana leaves (a) and banana pseudostems (b), which have different particle sizes.

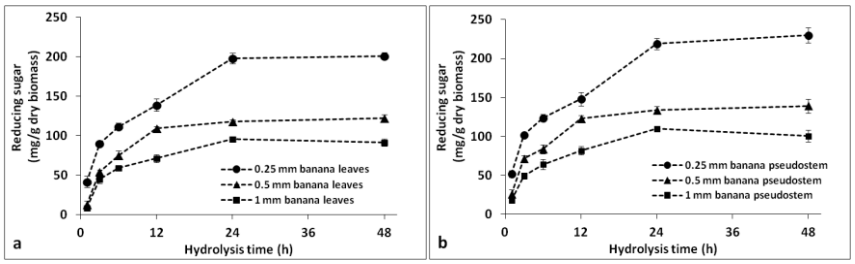

Figure 2. Time course of hydrolysis of banana leaves (a) and banana pseudostems (b), which have different particle sizes

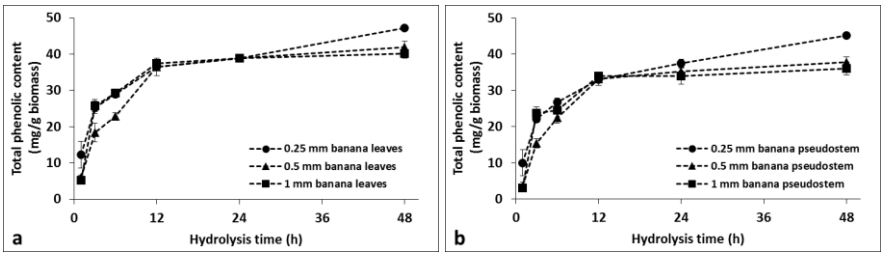

Figure 3. Total phenol concentrations in hydrolysates during hydrolysis of banana leaves (a) and banana pseudostems (b), which have different particle sizes

Additionally, the genotoxicity of hydrolysates were assessed by comet assay (Çavaş, 2007) using *Saccharomyces cerevisiae*. The comet assay was performed for determinated DNA damage in cells. Hydrogen peroxide was used as positive control. Standart fermentation medium was used as negative control. *Saccharomyces cerevisiae* cells were growth in medium prepared with hydrolysate for 24 h. The results showed that hydrolysates were not genotoxic on *Saccharomyces cerevisiae* cells.

Referances

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