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RECOVERY OF THERMOSTABLE CELLULASES FROM WOODLAND SOIL BY MEANS OF METAGENOMIC APPROACHES

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Cellulose is a major polysaccharide constituent of plant cell walls and one of the most abundant organic compound in the biosphere. It has enormous potential as a renewable source of energy and has attracted the interest of biotechnologist who wish to use it as a source of fuels and chemicals. Currently, most biofuel is in the form of ethanol generated from starch or sugar, but this can meet only a limited fraction of global fuel requirements. Conversion of cellulosic biomass, which is both abundant and renewable, is a promising alternative. Unfortunately, cellulose is naturally resistant to biological degradation because of its rigid structure and insolubility. The complete degradation of this polysaccharide into fermentable sugar involves the synergistic action of three different class of enzymes collectively known as cellulases: endo-β-1,4-glucanases, exo-β-1,4-glucanases, and β-glucosidases. Tolerance to high temperature is a desirable property of the enzymes, and the degradation of cellulose at high temperature shows several benefits, among which increased cellulose activity, lessened energy cost for cooling, and decreased risk of contamination. In this study we succeeded in isolating thermostable cellulases from woodland soil samples by means of metagenomic approaches. Based on a known thermostable endo-β-1,4-glucanases from Bacillus subtilis available online (A.N.: FJ464332) we developed primer pairs that were tested on the bacterial DNA directly recovered from the soil samples. Amplified fragments of the expected sizes were recovered from the gel, cloned and the single clones sequenced. Twenty five different cellulase sequences from B.subtilis were isolated. All the obtained sequences had nucleotide and aminoacid variations with respect to the online sequence. Ten out of twenty five sequence were cloned in expression vector pET28b and then successfully extracellularly expressed in Escherichia coli BL21. The activity and the thermostability of the enzymes were tested, two of the isolated genes show high performance at elevated temperature making them good candidates for biotechnological applications involving cellulose modification.