CLONING, CHARACTERIZATION AND EXPRESSION OF GENES CODING FOR CELLULASES.

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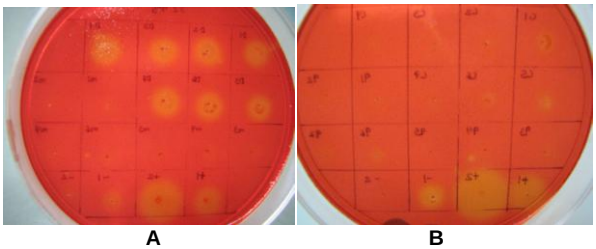
Introduction. Cellulose materials are particularly attractive as feedstock for renewable energy generation, for its low cost and for being the most abundant supply in nature (1). Some microorganisms associated with plant cell walls are able to use cellulose as the energy source, through the production of hydrolytic enzymes known as cellulases (2). Technological advances have enabled the production of synthetic cellulolytic enzymes, however, a very high price (3). Currently, in the biotechnological field models have been developed for obtaining expression of a high production of cellulases from microorganisms which naturally possess a gene coding for a cellulase or synthetic cellulolytic genes from optimized codon usage (4). Therefore, our goal was to clone genes coding for cellulases in *E. coli* expression system.

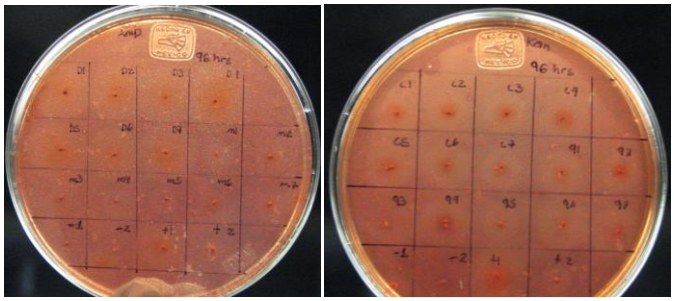
Methods. We isolated the cellulase gene CelD with endoglucanase activity of *Bacillus subtilis* ATCC 1774 and cloned into *E. coli* Mach1 T1. Synthetic genes Cel9A of *Phanerochaete chrysosporium* and CelC of *Neocallimastix sp.* having endoglucanase activity and the gene mfc cellulase of *Ampullaria crossean* having endoglucanase, exoglucanase and endoxylanase activity, were cloned into *E. coli* DH5α. 7 clones were selected from each gene were characterized with restriction enzymes and their activity was tested in M9 medium plates with 1% CMC or cristaline cellulose as sole carbon source, IPTG 1mM, ampicillin 100 µg/mL or kanamycin 30 µg/mL identifying three clones of each gene showed increased cellulolytic activity at 72 hours of incubation according to the diameter of the degradation halo showed (5).

Results. In the clones D3, D4 and D5 celD gene, degradation halo was observed of 0.8 mm. The clones C1, C5 and C6 of the synthetic gene CelC, 91, 93, 96 and m1, m4, m6 of the synthetic genes and gene mfc cellulase and Cel9A, respectively, showed degradation halo of 0.6 mm. According to the above results, the celD gene clones CelD of *B. subtilis* showed the highest degradation halo.



A B

Figure 1. Extracellular cellulase activity assay of the clones with CMC **A.** CelD gene clones (D1-D7) and mfc cellulase (M1-M7). **B**. CelC gene clones (C1-C7) and cel9A (91 - 97). +1 positive control strain Sol 2-5, +2 positive control strain is 1-6, -1 negative control strain *E. coli* Mach1 T1, -2 negative control strain *E. coli* DH5α.



A B

Figure 2. Extracellular cellulase activity assay of the clones with cristaline cellulose. **A.** CelD gene clones (D1-D7) and mfc cellulase (M1-M7). **B**. CelC gene clones (C1-C7) and cel9A (91 - 97). +1 positive control strain Sol 2-5, +2 positive control strain is 1-6, -1 negative control strain *E. coli* Mach1 T1, -2 negative control strain *E. coli* DH5α.

Conclusions. Cloning and characterization of the synthetic genes CelC, Cel9A and mfc cellulase carried out successfully and, although the celD gene clones of *B. subtilis* did not show the expected pattern in a cellulase degradation test clearly demonstrates its activity. Compared with other studies of cellulase gene expression, which is used LB medium and CMC 1% to test cellulolytic activity, in this work, a more specific test by using M9 medium and 1% CMC as the sole carbon source. cellulolytic cloning genes in strains of *E. coli* have allowed cellulolytic activity results in incubation times lower compared with native cellulolytic strains isolated, where a good halo assessing cellulose degradation times ranging between 168 and 360 hours.

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