903/A/Poster

Cloning of endoxylanase 23 (*exn23*) gene of *Trichoderma virens* using fusion PCR for expression in a bacterial system

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The hydrolysis of the hemicellulose matrix of lignocellulosic biomass, in order to release the cellulose fibers is a crucial step in second generation bio-ethanol production. Endo-1,4-β-xylanase (*exn*) is one of the main components in the hemicellulase enzyme complex. The main objective of this study was to modify the *Trichoderma virens* fungal endoxylanase gene and express it in a bacterial expression system *E. coli* BL21(DE3).

A recombinant genomic clone containing the endoxylanase gene in the vector pGEMT had been previously cloned in our laboratory. The *exn23* gene is 687 bp in size containing two exons and a single intron (114 bp) from 174 to 287 bp. Initially to remove the intron, fusion PCR was carried out as follows: DNA was extracted from the recombinant pGEMT plasmid containing the *exn*gene. Both exons were PCR amplified separately using the above clone as a template. The first exon was amplified using forward primer (EXN23FP) and a fusion primer (EXN23FS1) that contained a region complementary to the second exon, whereas the second exon was amplified using the reverse primer (EXN23RP) and a fusion primer (EXN23FS2) that contained a region complementary to the first exon. Subsequently, the amplified exons were used as templates and primers EXN23FP and EXN23RP were used for fusion PCR.

The above PCR fused fragment was subjected to TA cloning. Several recombinant clones were obtained and a clone designated *pEXN23* was sequenced. Analysis revealed *pEXN23* to be 100% similar to the coding region of the *exn23* gene of *Trichoderma virens*, indicating that the fusion PCR has successfully eliminated the intron.

Keywords: *Trichoderma virens,* second generation bio ethanol, hemicellulase, endo-1,4-β-xylanase , fusion PCR

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