

Amplification and cloning of D- amino acid oxidase; the enzyme catalyzes the antibiotic cephalosporin c to 7- amino cephalosporanic acid

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Background and Aims: The present study was designed to produce the recombinant d-amino acid oxidase, a catalyst widely used in pharmaceutical biotechnology in the yeast *Saccharomyces cerevisiae* as a host.

Methods: *Fusarium oxysporum* was used in this study due to its remarkable activity. This choice was determined using bioinformatics studies. Total RNA was extracted from the fungus and cDNA was synthesized. A pair of primer was designed for cDNA amplification of the d-amino acid oxidase. cDNA target was amplified using PCR. Cloning process including gene and plasmid digestion, digested fragment extraction from the gel and purified product ligation was performed and finally recombinant vector was transformed to the host yeast.

Results: Total RNA was extracted with 2000 µg / ml concentration and gel image was showed the respective bands of rRNA. Extracted RNA was used for cDNA synthesis and latter molecules were used as template for PCR. Two. restriction enzyme sites were designed in specific primers, which were *AscI* and *NotI*. The product containing whole length of d-amino acid oxidase cDNA with size of 1089bp was amplified successfully. The purified PCR product and p316TDH plasmid were double digested and then were ligated using T4 DNA-ligase enzyme. The shuttle vector p316TDH3DAO was transformed to the eukaryotic host yeast *Sacchromyces cerevisiae* and screening of transformed colonies carried out in the presence of an auxotrophic marker. **Conclusions:** The expression and production process of d-amino acid oxidase was performed using specific secretory peptide sequence of an alpha-factor. In this process, desired enzyme molecules were released in the medium directly without any requirement to use lysate cells, which are usually a painstaking work.

Keywords: D-amino acid oxidase; Recombinant protein; Cloning; PCR