E311 Cloning and Overexpression of Gene Encoding D-Arabinose-1,4-Lactone Oxidase in *Saccharomyces cerevisiae*

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D-Arabinono-1,4-lactone oxidase (ALO), which catalyzes the final step of D-erythroascorbic acid biosynthesis in *Saccharomyces cerevisiae*, consists of 526 amino acids. Four oligonucleotide primers were used to amplify two parts of ALO gene and the two products were directly ligated to pGEM-T Easy vector. Each subcloned plasmid was identified by automatic sequence analyzer. Using Sac I site belonging to each fragment, two subcloned inserts were excised and ligated to produce a clone containing the whole ALO gene. The recombinant *Escherichia coli* vector, pETALO was constructed for inducible expression of ALO gene. The cell BL21(DE3)pLysS was transformed with pETALO and expressed under control of IPTG. The recombinant yeast vector, pNVALO was constructed for inducible expression of ALO gene. The yeast cell L2612 having Gal+ phenotype was transformed with pNVALO and selected using *ura3* marker. Transformed L2612 cell was expressed under control of galactose in glucose-free medium.

E312 Purification and Characterization of Exopolygalacturonase from *Aspergillus* sp. W3

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Aspergillus sp. W3 producing exopolygalacturonase was isolated from bark. The exopolygalacturonase was purified by acetone precipitation, CM-Sepharose, DEAE-Sepharose, Sephadex G-150 column chromatography and preparative iso-electric focusing. The molecular weight of exopolygalacturonase was estimated to be 96,000 by SDS-polyacrylamide gel electrophoresis. The optimum pH and temperature was abuot 5.0 and 70℃, respectively. The K_m value of exopolygalacturonase on polygalacturonic acid was estimated to be 390 μg/mℓ. The isoelectric point(pI) of purified exopolygalacturonase was about 4.