

[BIO37]

Cloning and expression of the *Glomerella cingulata* cutinase recombinant gene in the yeast, *Pichia pastoris*

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Cutinase is produced by the phytopathogenic fungus, *Glomerella cingulata* (anamorph. *Colletotrichum gloeosporioides*) and is involved in enzymatic degradation of cutin during the penetration process of fungal appressoria through the plant cuticle. As a lipolytic enzyme, cutinase also has potential biotechnological applications e.g. in the detergent, dairy and oleochemical industries. Due to highly commercial potential for biotechnological applications, we proposed to express the recombinant cutinase by generating an efficient expression system in the methylotrophic yeast, *Pichia pastoris*. The intronless cutinase gene expressed in *P. pastoris* was constructed by SOE-PCR (Splicing of Overlapping Extension PCR) and was cloned in the intracellular expression vector, pPICZB. A myc-epitope fused to a polyhistidine-tag (His₆) was introduced at the C-terminus of the cutinase recombinant gene for ease of detection and purification. After *P. pastoris* transformation, colonies were screened for high level expression based on the ability of the colonies to grow at a high concentration of zeocin (1500 µg/ml). Cutinase expression was performed in the yeast using the methanol-inducible alcohol oxidase gene (*AOX1*) promoter in buffered minimal methanol medium (BMMY) using 1% methanol induction for four days. The expressed recombinant cutinase with a targeted protein size of 25 kD was identified by SDS-PAGE and western analyses. The protein was assayed for cutinase/esterase activity and subsequently purified by immobilised metal-affinity chromatography (IMAC) on AKTAPrime purification system (Amersham). The purification procedure resulted in 45% yield and four purification fold with the specific cutinase/esterase activity was at 21 U/mg.