

Expression of fungal Lignin Peroxidase in *Pichia Pastoris**

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Abstract

Lignin peroxidase is an enzyme which is potentially useful for processing of renewable resources as well as for enzymatic transformations of polyaromatic hydrocarbons. This work describes cloning and expression of lignin peroxidase in *Pichia pastoris* and the use of directed evolution to improve expression. The goal was to reduce the cost of enzyme production by eliminating fungal fermentations. The *lipH2* gene of *Phanerocheate chrysosporium* was mutagenized by PCR and subcloned into a series of *P. pastoris* vectors (Invitrogen) harboring a combination of native fungal and/or yeast promoters, consensus sequences and leader sequences. The vectors were transformed into several different strains of *P. pastoris*. The recombinant clones were screened for activity using a standard 2,2' azinobis-(3-ethylbenzothiazole-6-sulfonate) (ABTS) assay. We observed methanol-inducible expression in the host *P. pastoris* X33 strain transformed with the construct that contained yeast promoter, engineered yeast consensus sequence and native leader sequence. The activity and the amount of enzyme secreted into the medium was, however, very low. Attempts to improve expression via directed evolution (error-prone PCR) did not result in much improvement, indicating that *Pichia pastoris* may not be a suitable host for the enzyme.

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