

Cloning and expression of laccase gene in *P. pastoris* GS115

The 1.6 Kb codon optimized laccase gene of *Melanocarpus albomyces* was cloned downstream of signal peptide α - mating factor of pPIC9K vector for extracellular protein expression. The resultant recombinant vector was transformed into *P. pastoris* GS115, and the recombinant strain is designated as GS-COL. The recombinant strain is evaluated for the production of laccase in the shake flask.

Table 1: List of plasmid and strains used in this study

Plasmids or Strains	Genotype/Description
Plasmids	
pPIC9K	Vector for extracellular expression; Geneticin resistance
pPIC9K-COL	pPIC9K harbouring codon optimized laccase gene
Strains	
<i>E. coli</i> DH5 α	<i>F</i> - ϕ 80 <i>lacZ</i> Δ <i>M15</i> Δ (<i>lacZYA-argF</i>) <i>U169 recA1 endA1 hsdR17 (rK⁻, mK⁺) phoA supE44 λ - thi-1 gyrA96 relA1</i>
<i>P. pastoris</i> GS115	Commercial transformation host for cloning; his4 ⁻ , Mut ⁺
<i>P. pastoris</i> GS-COL	<i>P. pastoris</i> GS115 integrated with pPIC9K-COL

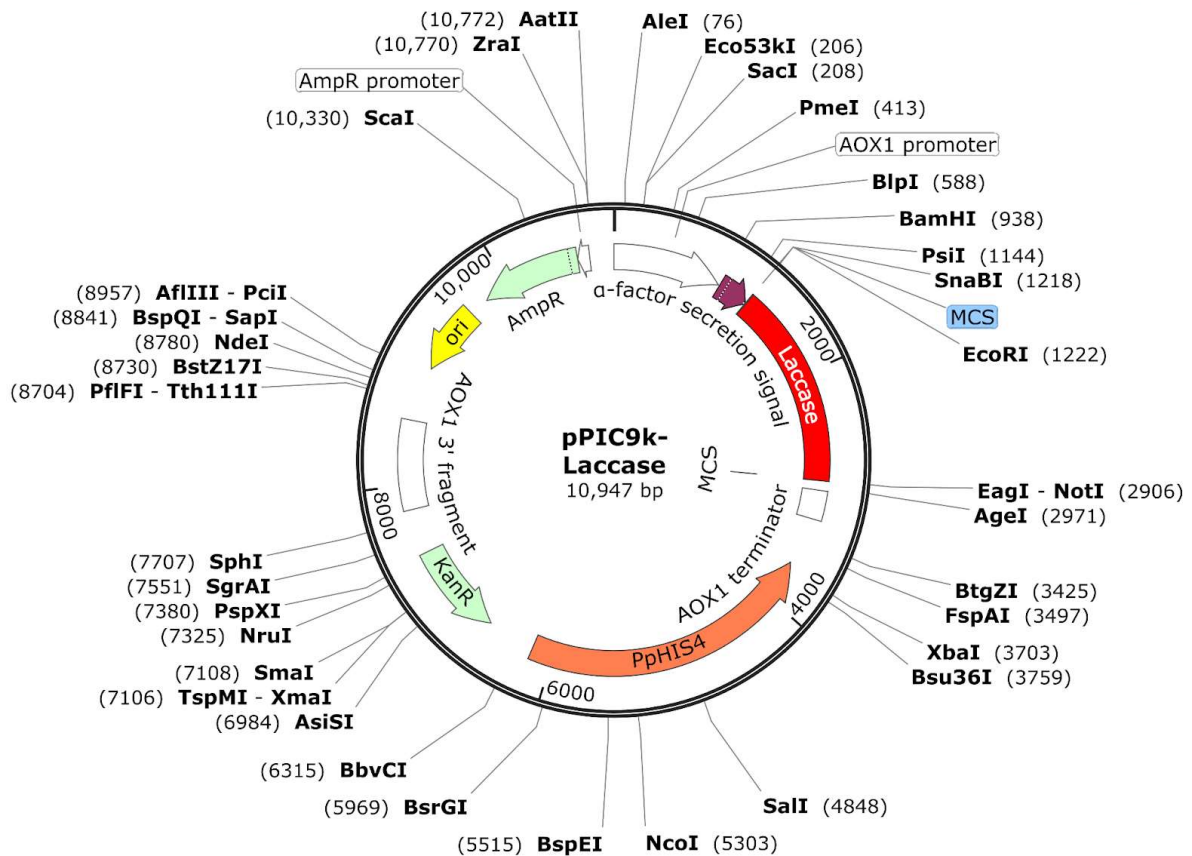


Fig : pPIC9k-Laccase plasmid BBa_K4077003

SHAKE FLASK STUDIES FOR LACCASE EXPRESSION

The recombinant strain GS115-COL was pre-cultured in BMGY medium, where Pure glycerol and crude glycerol, in both mediums the recombinant strain showed excellent growth displaying its ability to consume the impurities associated with crude glycerol. Further the pre-inoculum was transferred to BMMH medium and induced with 1% methanol.

The maximum production of laccase was found to be 9.75 U/ml at 48 h time interval with concomitant increase in cell concentration of 8.9 using pure glycerol based pre-inoculum (Fig 1A). Similarly using crude glycerol based pre-inoculum the maximum laccase and cell concentration was found to be 6.75 and 7.59, respectively (Fig 1B). The laccase clearly displayed a growth associated product. The crude extract will be used for the treating CSM.

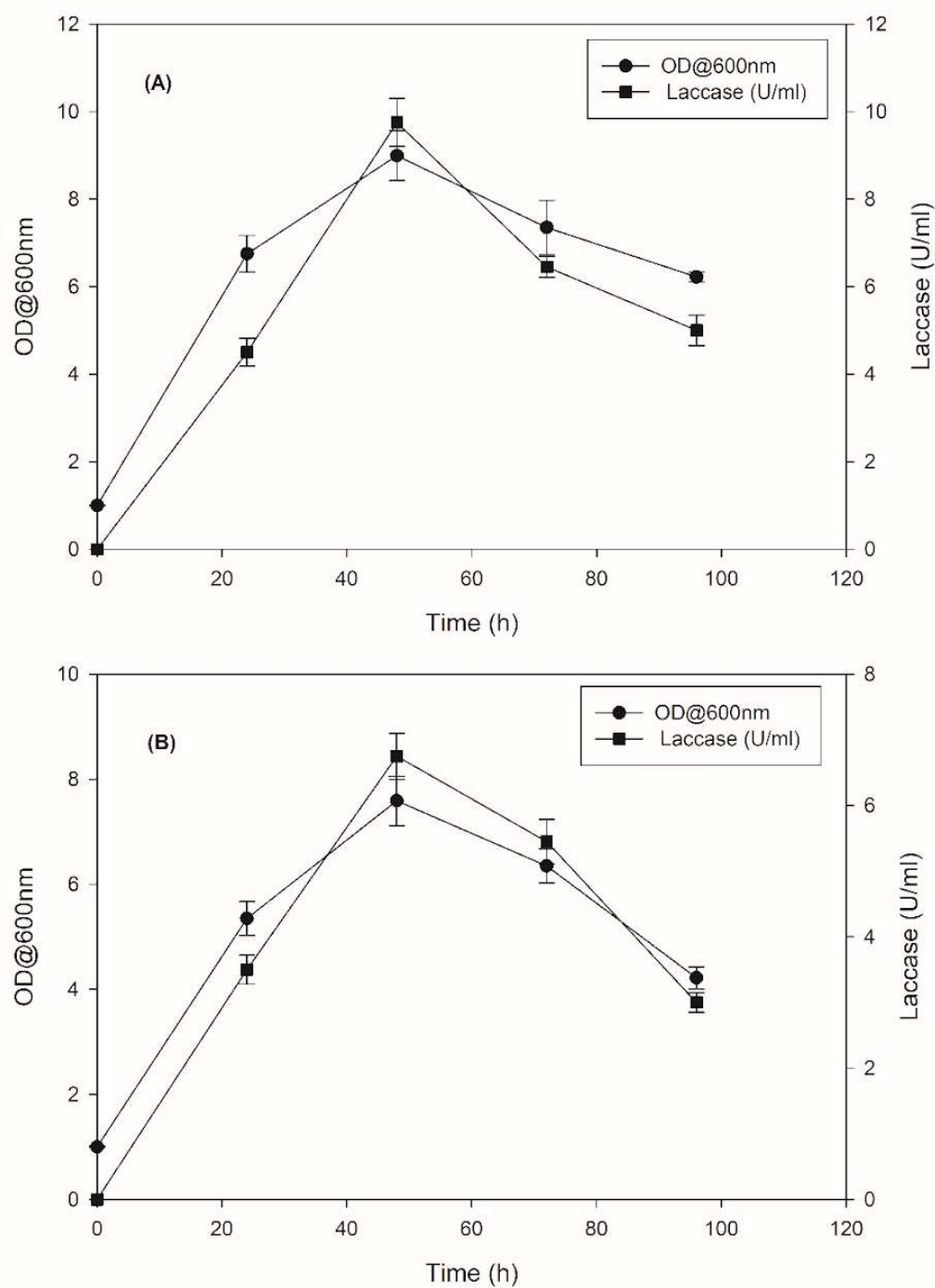


Fig 1. A. Growth and product profile of GS-COL strain grown in pure glycerol based pre-innoculum and BMMH medium with 1% methanol Induction. B. GS-COL strain grown in Crude glycerol based pre-innoculum and BMMH medium with 1% methanol Induction