INTRODUCTION

The Laccase enzyme: p-diphenol: oxygen oxidoreductase(EC 1.10.3.2)
- Multicopper enzyme belongs to blue oxidases
- Active site contains four copper ions (Cu²⁺) per molecule
- Broad substrate specificity. High oxidation capacity. Uses oxygen as final electron acceptor.
- Source: Majority of laccase have been isolated from higher fungi – Ascomycetes, Deuteromycetes and Basidiomycetes; especially white-rot Basidiomycete.
- Applications:
  - In pulp & paper, textile and cosmetic industries, for detoxification and decolorisation of sewage
  - In organic synthesis, for degradation of xenobiotics & bioremediation to create antimicrobial compositions
  - In production of wood-fiber plates, wood-blocks & cardboard without using toxic linkers
  - In detergents production
  - In elaboration of biosensors and cathodes of biofuel cells.
- Current production technologies & their limitations:
  - Most filamentous fungi produce several isoforms of laccase in high amounts.
  - However, an efficient production system at bioreactor level is still lacking.
- Our approach:
  - Over-expression of laccase in a suitable host
  - Reducing the cost of laccase production by optimizing the fermentation strategy.
  - Yeast are suitable host for heterologous protein production because of high capacity for growth, easy manipulation of unicellular organism & a eukaryotic organization enables post-translational modification.
  - Pichia is a well described & widely applied production system well known for high cell densities fermentation & hence higher productivity could be expected.

WORK DONE SO FAR

- Complete cDNA encoding laccase from Cypinus buperi was cloned, sequenced & expressed in Pichia pastoris under the influence of AOX1 promoter.
- Laccase secreted into the medium under the control of α-factor secretion signal of Saccharomyces cerevisiae
- Cultivation of the developed Pichia strain has been done at shake flask level on different media -YPD and BMMGY.

RESULTS

- Laccase activity was observed on induction with methanol.
- Maximum activities were observed on day 3 of the process on BMMGY medium (in shake flask)
  - Activity observed in the range of 100 IU/L (with ABTS as substrate).
- Laccase activity was fairly stable up to 100 h when stored at 4°C.
- Scale up was carried with a bench scale fermenter (5L) in a fed-batch mode
  - Total production of 1200 IU/L was obtained (fig 1).
  - Final biomass concentration was 270 g/L WCW (fig 1).
- Overall productivity of process was 300 IU/L.D

Fig 1: Process Chart
Fig 2: Effect of different temperatures on Laccase stability (above); biphasic trend of temperature stability (below)
Fig 3: Effect of Cu²⁺ on the stability of the protein

FURTHER PLANS

Experimental work is planned to achieve:
- Complete characterization of recombinant protein.
- Optimization of operation strategies to reduce the overall process time.
- An integrated approach for simultaneous fermentation & (semi) continuous product recovery.
- Improved stability of recombinant product.
- Minimal down-stream processing cost.

Fig 4. Schematic diagram of fermentation process with P. pastoris