Genetic Engineering of Yeast for Manganese Peroxidase Production

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Chemical, Biological and Environmental Engineering

Introduction

One of the measures being considered to mitigate global climate change is to sequester carbon. One form of biological sequestration is the growth of plants followed by the long term storage of carbonaceous biomass in forest or agricultural soils. Lignin, a complex aromatic biopolymer, is one of the main precursors of humus. Humus is the carbonaceous plant biomass derived material that can be quite stable for long periods of time in forest and agricultural soils, and forms the medium for biological soil carbon sequestration. It has been estimated that globally, soil carbon sequestration alone could offset as much as 15 percent of fossil fuel emissions.

Figure 1. MnP

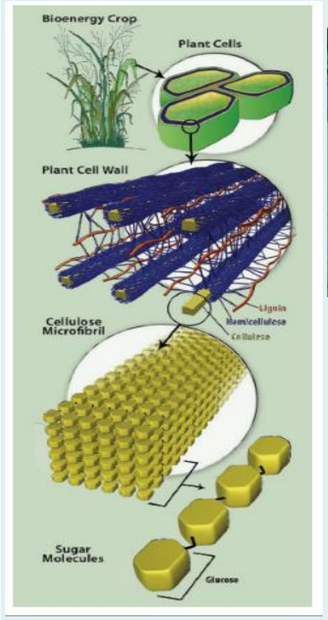

Figure 4. Structure of Biomass

Figure 2. White-rot Fungi

Figure 3. *Pichia pastoris*

Objective

MnP is one of the most important enzymes in lignin transformation in the subsurface. Significant quantities of pure MnP are required to investigate the fundamental mechanisms of lignin transformation . The objective of this research is to improve the production of recombinant manganese peroxidase (rMnP) (Figure 1) from the white-rot fungus *Phanerochaete chrysosporium* (Figure 2) in the yeast *Pichia pastoris* (Figure 3) by inserting a heme transporter.

Heme Transport

MnP contains a heme (Figure 5) group that is essential for degradative activity. A major discrepancy between the expected heme requirement for the amount of rMnP produced and the actual concentration required to maximize rMnP production was observed. These results suggest problems with heme transport, insertion, or folding during rMnP synthesis in the yeast.

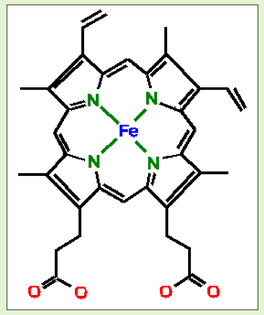

Figure 5. Structure of Heme

GGNNCNGCCGGCCGTCTCGGATCGGTACCATGCTATCGTCTGTGTTTTTTTC
AATATTAACATTACTACTTTCTTGCTTTGTATCAACTACTGAAGCCAGAAGGA
AGCTTTCGGCTACTTCTTTAGTCACATGTATGGATAATTCACAAATTTCTCCT
AGTTATTTCAATGTTACATTCAATCCCGATGATAGATCTTTAAGGTATGCCCTT
GATTTAACTACTTCAATTAGTGCATATATTACTGCTCACGTTCAAGTTTATGCT
TATGGGTTTTTAATTATTGAAAAAGATATAAATGTTTGTTCCTTGGGTTGGAA
ACAATTTTGTCCAATTTTCCCTGGTGCTTTACAAATTGATTCTGTTCAATATAT
TAGTTCGGAATACACTAAACAAATCCCTGGTATTGCTTATCAAGTGCCCGAT
ATTGATGCTGTGGTAAAACTTGTTGTTAAAGAAAGAGATTCAGGTGATACAT
TATCTTGTTTACAAGCCAATTTTTCCAATGGTAAAACTATTAGTCAAACCGGA
GTTAAATGGGCCACCGCATGTATTGCTGGTTTAGGATTGATTGTGGCTGCTA
TGTTGTCTACTTTTGGAAATTCTAATGCTGCTTCCCACATTTCGGCTAATTCA
TTATCACTTTTCCTTTATTTCCAAAGTGTTGTTGTTGTTTCAATGCAAGCAGT
TGAAAGAGTCCCACCAATTGCCAGTGCTTGGTCAGAAAATTTAGCTTGGTC
AATGGGATTAATTCGTATTCAATTTATGCAAAATATTTTCCGTTGGTATATTCA
ACTGACTGGAGGTTCACCAACAACATATTTTATTTCGTCAACCAAACAATTT
TAGTACAAAAACACAAGAGATCTTTAGATTATGTTGCTGGTGTGGCTAAAAG
ATCTTTAGAATATGCTTTACGTTCACATAGTAATTTATATGTGTTGCGTGGNAT
TAAAAGAATTGGGTATAATTCCAAATTGAACCAACATCNATNNNCNCTGGAT
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Figure 6. Sequence data indicting presence of CaFLC1 gene cloned into plasmid (pGAPZA)

Cloning Heme Transporter

A recently discovered gene (CaFLC1), secretion-targeted heme/flavin adenine dinucleotide transporter, has been PCR amplified from *Candida albicans*, and cloned into the *Pichia* expression vector PGAPZ Between the multicloning sites KpnI and ApaI (Figure 9). The CaFLC1 gene was then inserted into *Pichia* via electroporation and homologous recombination. It is hypothesized that this gene product will aid in heme transport, insertion, and folding of the MnP during synthesis.

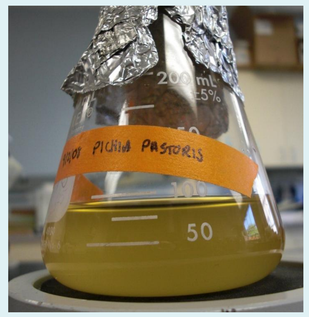

Figure 7. Shake-flask with *Pichia pastoris*

CaFLC1 Gene

CaFLC1 has 9 to 10 transmembrane domains suggesting it is an integral membrane protein that functions as a transporter. The protein is implicated in transport of both FAD and heme. In addition to requiring heme, MnP contains five disulfide bridges, which require FAD for proper formation, making CaFLC1 in promising candidate to improve active MnP expression.

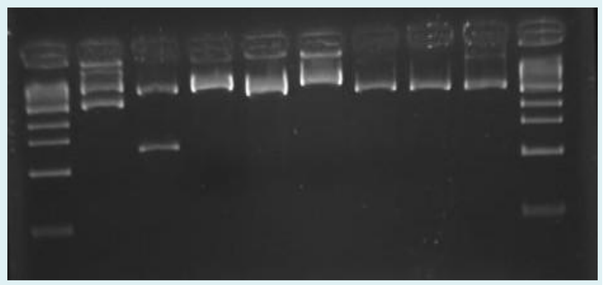

Figure 8. Gel electrophoresis indicting presence of CaFLC1 gene cloned into plasmid (pGAPZA)

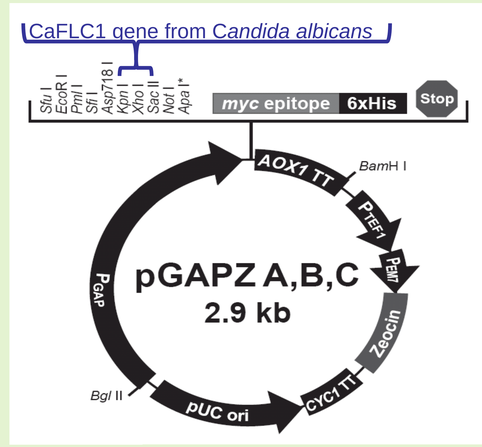

Figure 9. *Pichia* vector for cloning gene encoding a secretion-targeted heme/flavin adenine dinucleotide transporter (CaFLC1).

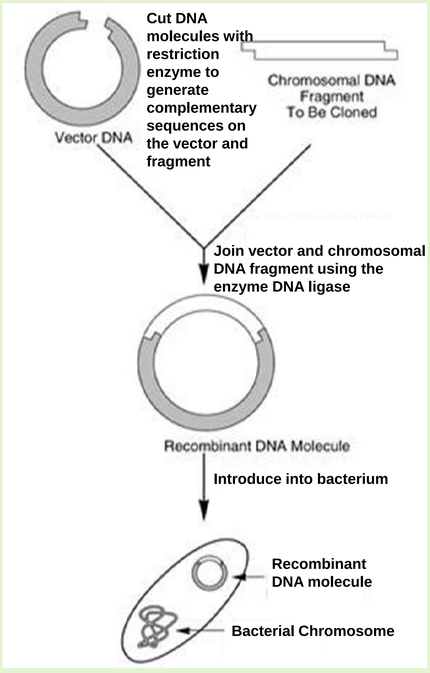

Figure 10. Standard cloning technique

Acknowledgements

This work was supported by The Subsurface Biosphere Initiative (SBI).