LACCASE AS A TARGET FOR DECREASING LIGNIN CONTENT

Peter R. LaFayette', Clayton L. Rugh[2], Scott A. Merkle[2], Karl-Erik L. Eriksson', & Jeffrey F. D. Dean'

Department of 'Biochemistry and Molecular Biology and [2]Daniel B.Warnell School of Forest Resources, University of Georgia, Athens, GA 30602-7229

Abstract. Studies show that laccases (p-dipheno1:0$_2$ oxidoreductase, EC 1.10.3.2) are specifically expressed in lignifying xylem from a variety of vascular plants where they may play a role in the deposition of lignin. Laccases are members of a highly conserved class of metalloenzymes, the "blue" copper oxidases, which includes ascorbate oxidase and ceruloplasmin. Plant laccases can be fractionated into two isoform classes on the basis of their isoelectric point. These different pl forms may be involved in discrete functions in the cell. cDNAs encoding laccase have been isolated from libraries prepared from 1) suspension-cultured cells of *Acer psuedoplatanus* (sycamore maple) and 2) cambial/lignifying zone tissue of *Liriodendron tulipifera* (yellow-poplar). The *A. psuedoplatanus* full-length cDNA clone encodes an acidic form of laccase and represents an highly abundant message in the suspension-cultured system. The *L. tulipifera* cDNA clone encodes a basic form of the enzyme and its message level is comparatively low. Although the cloned genes show more homology to one another than with any other gene sequence in the gene database, their sequences are only 45% identical (65% similar) at the amino acid level and they do not cross-hybridize at low stringency. As would be expected, the amino acid ligands for binding of the catalytic copper atoms are completely conserved between these two genes and most other blue copper oxidases. To test the hypothesis that laccase is involved in the deposition of lignin, we prepared an antisense construct of the yellow-poplar gene which contained the 5'-untranslated region and 12% of the amino-terminal coding sequence. The antisense laccase sequence was fused to the nominally constitutive CaMV35S viral promoter. The construct was introduced into embryogenic cultures of yellow-poplar by microprojectile bombardment, and over 60 kanamycin-resistant cell lines were recovered. These cell lines are currently being analyzed for antisense gene expression. This research was supported by Georgia Pulp and Paper Consortium grant PP96-FS3.

Keywords: *Acer psuedoplatanus, Liriodendron tulipifera,* genetic engineering, laccase, lignin, pulp bleaching.