Phenoloxidase Production by Amylostereum areolatum: Applying an Exotic Invasive to Bioresource Conversion

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Phenoloxidase activity has been detected on solid cultures of A. areolatum via oxidation of chromogenic substrates: guaiacol, syringaldazine, and ABTS (2,2’-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)).

The two common phenoloxidases secreted by white-rot fungi are peroxidase and laccase. Laccase and peroxidase share common chromogenic substrates, but have different cosubstrates. Peroxidases oxidize their substrates in the presence of hydrogen peroxide. Laccase only requires the presence of molecular oxygen, which is ubiquitous under typical laboratory conditions.

We have demonstrated that oxidation of ABTS and guaiacol in liquid media takes place in the presence of catalase, a peroxide scavenger. Oxidation in the absence of hydrogen peroxide (peroxidase cosubstrate), is evidence that we are recognizing laccase activity.

Purification Scheme

The fungal protein mixtures were fractionated by partial purification in a three-step process. Crude liquid culture filtrate was ultrafiltered and concentrated using a 30 kDa MWCO membrane, followed by ammonium sulfate precipitation, and a final concentration with a combination of ultrafiltration and centrifugal concentration (10 kDa MWCO):

- Partial Purification
- Concentration (10 kDa MWCO)
- Partial Purification
- Concentration (10 kDa MWCO)

PAGE: Enzyme Activity

Phenoloxidase zymogram of the partially purified A. areolatum laccase. Arrows indicate molecular weight in kDa. SDS-PAGE (Tris-tricine system), run at constant voltage (30V) until sample entered the separating gel, then 150V until dye electrophoresed off the end of the gel. Phenoloxidase activity detected with 1,8-diaminonaphthalene staining (2 mM DAN in 1% DMSO/50 mM sodium acetate, pH 5).

PAGE: Total Protein

Coomassie-Stained Zymogram Gel Containing the Partially Purified A. areolatum Laccase. Samples not boiled prior to loading gel. Arrows indicate molecular weight in kDa. SDS-PAGE (Tris-tricine system), run at constant voltage (30V) until sample entered the separating gel, then 150V until dye electrophoresed off the end of the gel.

Further Work:

A final column separation will likely serve to fully purify the enzyme. Once accomplished, kinetic data can be collected. Purified laccase from A. areolatum is expected to have utility in the oxidation and breakdown of recalcitrant biopolymer (lignin), thus streamlining the process of converting the remaining cellulose to biofuel (ethanol). This approach avoids additional chemical inputs to the environment, and energetic output in the form of burning fossil fuels.

The laccase has been shown to oxidize resveratrol, suggesting a role for the laccase in detoxification of plant defense compounds. Learning what extent the enzyme is capable of oxidizing pinoresinol, a known defense compound in pine, would aid in elucidating the pathogenic mechanism of A. areolatum.

References