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# ENZYME ACTIVITIES OF *Pleurotus sajor-caju* DURING SOLID SUBSTRATE FERMENTATION OF SAGO HAMPAS

By

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## ABBREVIATIONS

| | |
|---|---|
| ADF | Acid Detergent Fiber |
| ANOVA | Analysis of Variance |
| AOAC | Association of Official Agricultural Chemists |
| BOD | Biological Oxygen Demand |
| CMCase | Carboxymethyl cellulase |
| C : N | Carbon : Nitrogen |
| COD | Chemical Oxygen Demand |
| CTAB | Cetyl Trimethlammonium Bromide |
| DNS | Dinitrosalicylic Acid |
| EDTA | Ethylenediamine Tetra Acetic Acid |
| FPase | Filter Paper Hydrolysis Activity |
| *g* | Relative Centrifugal Force |
| $K_m$ | Michaelis constant |
| h | hour |
| L | Liter |
| MW | Molecular Weight |
| MWCO | Molecular Weight Cut Off |
| nm | nanometer |
| PDA | Potato Dextrose Agar |
| psi | Pounds per Square Inch |
| rpm | Revolution per Minute |
| SSF | Solid Substrate Fermentation |
| t/day | tonnes/day |
| $V_{max}$ | Maximum Forward Reaction Velocity |
| (v/v) | Volume per volume |
| (v/w) | Volume per weight |
| (w/w) | Weight per weight |
| λ | Wavelength |
| µmole | Micromole |

# ABSTRACT

Solid substrate fermentation (SSF) of sago pith residue, known as 'hampas' with the gray oyster mushroom, *Pleurotus sajor-caju* for enzyme production was carried out. Excellent growth of *P. sajor-caju* was noted on 'hampas' supplemented with 0.38% urea, 0.2% $KH_2PO_4$ and 0.05% $MgSO_4.7H_2O$ over an SSF period of 21 days. Endocellulase, filter paper hydrolysis activity, β-glucosidase, xylanase and laccase were detected in *P. sajor-caju* cultures. Maximum laccase and xylanase activities of 10.6U/g and 10.1U/g, respectively, were noted after nine days of SSF. The cellulose degrading enzymes; endocellulase, filter paper hydrolysis activity and β-glucosidase were less pronounced with activities ranging from 0.04 to 2.85 U/g 'hampas'.

Further investigation showed that the laccase and xylanase activities were influenced by the inoculum age and density over a 12 day period of SSF. With the 4-week old inoculum, laccase activity increased by about 3 to 12-fold compared to the activity with 2-week old inoculum. With the 4-week old inoculum, xylanase activity increased by only 1 to 2-fold compared to the 2-week old inoculum throughout the SSF. The α-amylase and glucoamylase activities were insignificant and together with microscopic examination of the spent 'hampas', it was inferred that the starch component of 'hampas' was not utilized by *P. sajor-caju*.

With the 4-week old 10% inoculum density, maximum laccase activity of 17.7U/g 'hampas' was recorded after six days of SSF. This amount could be almost doubled by addition of either 0.2 mM vanillin or ferulic acid. The apparent $K_m$ and $V_{max}$ values of crude laccase were 0.073 mM and 0.962 U/min., respectively. The laccase of *P. sajor-caju* was 100% thermostable at 30-55°C for 2 h and stable at a pH range of 4.5-9.5 at 30°C. A 14% loss in lignin was observed in the 'hampas' supplemented with inducers. The cellulose/lignin ratio increased significantly from 2.74 in control to 3.3 when 0.2 mM of either vanillin or ferulic acid was added to 'hampas'. Partial purification of the induced culture extract gave 81% laccase yield with a 3-fold purification.

On the basis of these results, the degradation by *P. sajor-caju* and possible utilization strategies of 'hampas' were proposed and discussed.

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