Formulation of a Cost-Effective Rice Straw-Derived Medium and an Attempt to Create a Recombinant *Trichoderma reesei* Strain to Enhance Its Cellulase Activity in Search of Bioethanol

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Oryza sativa L. (Rice) straw is a byproduct resulted while harvesting rice from paddy. *Trichoderma reesei* secretes numerous extracellular cellulases and hemicellulases, and degrades lignocellulosic matter giving fermentable reducing sugars. Linking these two 'assets' for bioethanol production this study proposes an economical and eco-friendly solution for exacerbating energy crisis. Therefore, the study aimed to obtain high yields of fermentable sugars, thus bioethanol, by cloning and expressing the lignin degrading laccase gene of the white rot fungus, *Rigidoporus microporus* in a highly cellulolytic *T. reesei* strain to further enhance its cellulase activity, and to induce the expression of the above cellulase and laccase genes by formulating a cost-effective rice straw-derived medium. Endo-1,4-beta-D-glucanase activity of the initial *T. reesei* strain on differentially formulated rice straw-derived culture media was studied against the standard medium, carboxymethyl cellulose. Reducing sugar concentrations caused by secreted cellulases in each culture medium were recorded at 24 hour intervals with 3,5-Dinitrosalicylic acid assay at 540 nm, converted into respective cellulase concentrations, and analyzed statistically. To clone the laccase gene by *Agrobacterium*-mediated pBI121 transformation, the *R. microporus* transcriptome was amplified using a gradient polymerase chain reaction with laccase gene-specific primers following reverse-transcription, and requires further optimization. Results suggested alkaline and acid pretreatments on mechanically processed straw were adding ($p<0.05$) to cellulolysis equally, since their cellulase concentrations were not significantly different. According to paired samples tests against the standard medium, Tween-80 was identified as a cellulase inducer ($p=0.051$), whereas gelatin ($p=0.657$) and L-ascorbic acid ($p=0.920$) alone could not significantly increase cellulase production. The medium containing alkaline-pretreated straw in a mineral salt medium with Tween-80, L-ascorbic acid and gelatin rendered the highest supernatant endo-1,4-beta-D-glucanase activity (3.599 Units/ml) on the 7th day after inoculation with *T. reesei*, and presents a promising medium for cellulase and laccase induction.

Keywords: Cellulolysis, Saccharification, Tween 80, Laccase, Gelatin, L-ascorbic acid