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Utilization of Paddy Husk for Tannase Production under Solid State Fermentation

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The present work has been taken up with a view of exploring the possibilities of using Agriculture by products with Tannic acid as a source for the production of tannase and optimizing condition require to get maximum production The fungus *Aspergillus oryzae* produced tannase from the cheap source of Paddy Husk on solid state fermentation. The crude tannase activity was found to be 9.5 U/g/min after Purification of Tannase by ammonium sulphate Precipitation 14.9 U/g/min, Dialysis 23.5 U/g/min and Column chromatography 28.3 U/g/min which increase the enzyme activity.

Key words: Tannase, Tannic acid, Fermentation, Purification, Paddy Husk powder.

Tannase (tannin acyl hydrolase, E.C.3.1.1.20) is an inducible, extra cellular hydrolase enzyme that catalyzes the breakdown of ester and depside bonds present in hydrolysable tannins or gallic acid esters, liberating glucose and gallic acid (GA).Tannin acyl hydrolase commonly called tannase is produced by a number of microorganisms like fungi -*Aspergillus, Penicillium, Rhizopus* sp, yeast - *Candida* sp and bacteria - *Bacillus* sp[1,2]. Rice husk, an agro waste, is cheap and abundantly available in India. Production of rice in India in the year was 88.25 million tones. Rice husks, a major part of the rice crop, are wasted or are largely underutilized. However, many researchers have tried to use Paddy Husk for energy, ceiling boards and ruminant feed. This study aimed to investigate the bioconversion of Paddy Husk powder for tannase production

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MATERIALS AND METHODS

Production and purification of tannase under Solid state fermentation

The experiment was conducted in 250 ml Erlenmeyer flask and it contains five gram substrate of Paddy Husk and 10 ml of salt solution[3]. The composition of the salt solution was NH_4NO_3 0.5 %, NaCl 0.1 %, $MgSO_4 \cdot 7H_2O$ 0.1 % and Tannic acid 4% at pH =5.5. The contents were sterilized by autoclaving at 121ºC, 15lbps for 20 min. The cooled sterilized solid substrate was inoculated with 1 ml of the *Aspergillus oryzae* MTCC 634 spore inoculums, mixed properly and incubated at 30 °C for 96 hrs.

The fermented substrate was homogenized with 0.05M Citrate buffer, pH 5.0. Then it was centrifuged at 8000 rpm for 20 min at 4 °C. The supernatant was collected and estimate the crude tannase activity following the procedure of Sharma *et al.*[4]. Then the crude tannase was precipitated with 80% solid ammonium sulphate. The precipitated enzyme was dialyzed against citrate buffer for overnight at 4ºC. The dialyzed

sample was again purified with DEAE sephadex column chromatography.

RESULTS AND DISCUSSION

The fungi *Aspergillus oryzae* MTCC 634 produced extracellular tannase under solid state fermentation using paddy husk as a substrate. The crude tannase activity was found to be 9.5 U/gm/min. The activity was increased to 14.9 U/gm/min at 80% of ammonium sulphate purification. The increased activity of tannase on successive purification was found to be 23.5 and 28.3 U/gm/min respectively given in Fig 1.

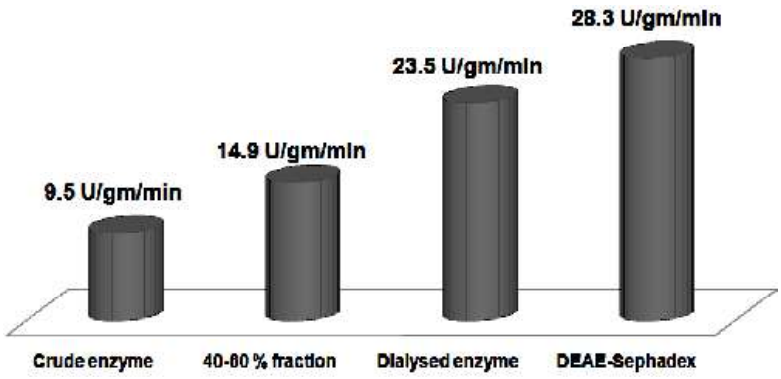

Fig. 1. Tannase activity on various purification steps

CONCLUSION

The present research indicated that the production of tannase using Paddy Husk as a substrate on solid state fermentation. From this research we have found that the Paddy Husk with addition of tannic acid is a suitable substrate for the production of tannase.

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