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20th Global Congress on Biotechnology

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3rd International Conference on Enzymology and Molecular Biology

March 05-07, 2018 London, UK

J Biotechnol Biomater 2018, Volume 8 DOI: 10.4172/2155-952X-C2-092

## Studies on optimization of improved amylases developed by protoplast fusion of Aspergillus species

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Improved amylase producers were developed by the fusion of protoplasts from two indigenous amylase-producing *Apergillus* species. Twenty randomly selected, regenerated fusants were screened for improved amylase activities. Selected fusants were used for further comparative studies with the parent isolates. Effect of incubation period on amylase activities of parent and fusant isolates were studied by assay of crude enzymes produced by solid state fermentation on rice bran and used for cassava starch hydrolysis. The interaction between temperature, pH and enzyme types (parental and selected fusants) was studied and optimized using the central composite design (CCD) of the response surface methodology (RSM). Fusant designates, T5 T13 and T14 were selected for improved activities over parents and were used in further comparative studies. Assay of amylase activities of randomly selected regenerated fusants at room temperature and at 80°C showed designates, T5 (920.21 U/ml, 966.67 U/ml), T13 (430 U/ml, 1011.11 U/ml) and T14 (500.63 U/ml, 1012.00 U/ml) as preferred fusants. Amylases produced by the fusants were observed to be active over the range of pH studied. Analysis of the sequences generated suggests the occurrence of genetic recombination in selected fusants, fusants T13 and T14 had optimum amylase production at 72 hours, while parents TA, TC and fusant T5 produced amylase maximally after 96 hours of incubation. Optimum amylase activity was observed from amylase from fusant T5 at pH 4 and temperature of 40°C. These results show the efficiency of protoplast fusion technique in strain development for improved amylase activity.

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