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Research Article

AMYLASE PRODUCTION BY *ASPERGILLUS NIGER* IN SOLID STATE FERMENTATION USING AGRO-INDUSTRIAL SUBSTRATES

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ABSTRACT
Solid state fermentation holds tremendous potentials for the production of the enzyme amylase by *Aspergillus niger*. Different solid substrates like rice bran, wheat bran, black gram bran, coconut oil cake, gingerly oil cake and groundnut oil cake are rich in starch. These agro industrial residues are cheap raw materials for amylase production. *Aspergillus niger* was identified to be the best producer of amylase. Among four substrates screened; coconut oil cake (COC) gave highest enzyme production 4.08 x 109 moles), which was almost two times higher than that produced by other substrates. Coconut oil cake has been a highly reported substrate producing promising results, among the various agro-industrial substrates used. Widespread suitability of COC may be due to the presence of sufficient nutrients and its ability to remain loose even in moist conditions, thus providing a large surface area. Oil cake especially coconut oil cake are rich source of starch. Oil cakes are rich in fiber, protein and energy contents. These agro industrial residues are cheap raw materials for amylase production. Extracellular production of α-amylase by *Aspergillus niger* under solid state fermentation using coconut oil cake as substrate was evaluated.

Keywords: *Aspergillus Niger; α-amylase; Coconut Oil Cake; Enzyme Activity*

INTRODUCTION
Amylases are a group of hydrolases, widely distributed in nature and can be extracted from plants, animals and microorganisms (Pandey *et al.,* 2005; Reddy *et al.,* 2003). Among these fungal and bacterial α-amylases have predominant applications in the industrial sector.
Fungi are used mainly for the production of amylase because of its bulk production capability and ease of manipulation. Aspergillus and Rhizopus species are mainly used as a source fungal α-amylase (Pandey *et al.,* 2005).
Recently both submerged fermentation (SMF) and solid state fermentation (SSF) are carried out for the fugal production of amylase (Miranda *et al.,* 1999; Norouzian *et al.,* 2006; Bhatnagar *et al.,* 2010). In cause solid state fermentations the extraction procedure are cheaper (Kumar and Duhan, 2011). Agro-industrial waste has been reported to be good substrate for the production of α-amylase because synthetic media used has been costlier (Miranda *et al.,* 1999; Norouzian *et al.,* 2006; Bhatnagar *et al.,* 2010). Crop residues such as bran, husk, bagasse and fruits seeds have been used as a potential raw material in enzyme production because they provide an excellent substratum for growth and supply the essential nutrients to them (Pandey and Soccol, 1998; Pandey *et al.,* 2000a,b,c,d,e).
Coconut cake has been used as a potent substrate for production of amylase by *Aspergillus niger* in solid-state fermentation because as it rendering both carbon and nitrogen (Benjamin and Pandey, 1997). Rice bran contains fat, crude fiber, carbohydrate (34%) and protein and its moisture content in the range 7-13%.
It also contains minerals such as potassium, phosphorus and calcium in considerable amounts. Therefore, rice bran potentially can be used as a substrate without further supplementation (Aikat and Bhattacharyya, 2001; Amissah *et al.,* 2003).
Rice husk used for enzyme production by solid state fermentation contains cellulose (35%), hemicellulose (25%), and lignin (20%) and it is a good source of lignocellulosic biomass that may be utilized as raw material (Abbas and Ansumali, 2010).
Thus the present study screens a variety of easily available and inexpensive agro-industrial substrate for the production of α-amylase using *Aspergillus niger* under solid state fermentation.

MATERIALS AND METHODS

Substrate Selection and Sterilization

Rice bran and rice husk were collected from a rice mill from Ramapuram, Kottayam district of Kerala, India. Coconut oil cake was procured from a local mill located at Udambannor, Idukki district of Kerala, India and ground nut was purchased from a provisional store. All the samples are subjected to steam sterilization for an hour at 121°C.

Isolation and Identification of Aspergillus niger

The fungus was isolated from moistened bread by serial dilution and spread plate technique. The different fungal strains were selected and subjected to lactophenol cotton blue staining and from the morphology and spore colour the culture was confirmed as *Aspergillus niger* (Aneja, 2003).

Determination of Amylase Activity

Aspergillus niger isolate was tested for amylase production by starch hydrolysis.

Figure 1: Various agro-industrial bye products used as substrates for the production of amylase, coconut oil cake (top left); ground nut oil cake (top right); rice bran (bottom left); rice husk (bottom right)

Enzyme Production by Solid State Fermentation

Aspergillus niger was subjected to solid state fermentation in different substrates like rice husk (S1, S2, S3), rice bran (S4,S5,S6), coconut oil cake (S7,S8,S9) and groundnut oil cake (S10,S11,S12) which was used as solid substrates for SSF. Each substrate was taken in about half inch thickness in all the bottles and hydrated with 25ml of basal salt solution and adjusted with moisture content from 43-81%. 1% of inoculums was inoculated after sterilization and incubated at room temperature for six days.

Enzyme Extraction Assay

25 ml of 0.1M phosphate buffer saline (pH 7) was added to each of the inoculated substrate beds and was vigorously shaken in rotary shaker for 15 minutes at 120rpm. The mixture was filtered through cheese cloth and centrifuged at 8000rpm at 40°C for 15min. The supernatant was filtered through cheesecloth

and the filtrate was used as the crude enzyme preparation. Enzyme amylase was assayed by Dinitrosalicyclic acid method.

Statistical Analysis

The results were analyzed and descriptive statistics were done using SPSS 12.0 (SPSS Inc., an IBM Company, Chicago, USA) and graphs were generated using Sigma Plot 7 (Systat Software Inc., Chicago, USA).

RESULTS AND DISCUSSION

Evaluation of Agro-industrial Residues as Substrates for SSF

Enzyme activity in the extracted enzymes from different substrates was determined by DNS assay and the results of the same can be seen in table 1-4 and chart 1-4. The histogram in chart 5 shows a comparison between the alpha amylase activities in the bottles containing different substrates used in the present study. All the four were found to be good substrates as the alpha amylase activity was seen in all the four flasks. It can be seen that maximum amylase activity was seen when coconut oil cake was used as substrate followed by ground nut oil cake, rice bran and rice husk enzyme activity was maximum in the bottle containing coconut oil cake as substrate and it was found to be 4.08 x10-9mols/ml followed by ground nut oil cake (2.19 x10-9mols/ml), Rice husk (1.19 x10-9mols/ml) and rice bran (0.64 x10-9 mols/ml).

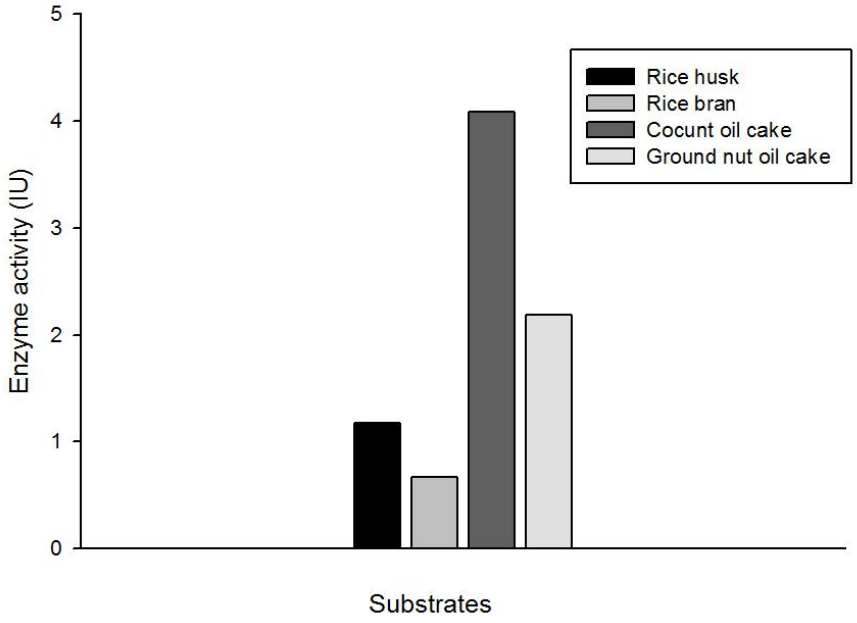

Figure 2: Enzymatic activity of various agro-industrial bye products (rice husk, rice bran, coconut oil cake, ground nut oil cake) used as the substrates for production of amylase

Table 1: Activity of amylase produced from different substrates (rice hush, rice bran, coconut oil cake, ground nut oil cake; n=4) three trials and the total trial average

	Rice husk	Rice bran	Coconut oil cake	Ground nut oil cake
Trial 1	1.23 ± 0.26	0.64 ± 0.01	4.25 ± 0.08	2.35 ± 0.13
Trial 2	1.23 ± 0.26	0.72 ± 0.08	4.01 ± 0.06	2.22 ± 0.06
Trial 3	1.10 ± 0.22	0.67 ± 0.04	3.98 ± 0.03	2.00 ± 0.10
Trial average (total)	1.18 ± 0.04	0.67 ± 0.02	4.08 ± 0.08	2.19 ± 0.10

Numbers represent means ± one standard error (SE) of the mean

Conclusion

Solid state fermentation holds tremendous potentials for the production of the enzyme amylase by *Aspergillus niger*. Different solid substrates like rice bran, wheat bran, black gram bran, coconut oil cake, gingely oil cake and groundnut oil cake are rich in starch. These agro industrial residues are cheap raw materials for amylase production. *Aspergillus niger* was identified to be the best producer of amylase.

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REFERENCES

Aikat K and Bhattacharyya BC (2001). Protease production in solid state fermentation with liquid medium recycling in a stacked plate reactor and in a packed bed reactor by a local strain of *Rhizopus oryzae*. *Process Biochemistry* **36**(11) 1059-1068.

Abbas A and Ansumali S (2010). Global potential of rice husk as a renewable feedstock for ethanol biofuel production. *Bioenergy Research* **3**(4) 328-334.

Amissah JGN, Ellis WO, Oduro I and Manful JT (2003). Nutrient composition of bran from new rice varieties under study in Ghana. *Food Control* **14**(1) 21-24.

Aneja KR (2003). *Experiments in Microbiology, Plant Pathology and Biotechnology,* fourth edition, (New Age International (p). Ltd.) New Delhi 607-615.

Bhatnagar D, Joseph L and Raj RP (2010). Amylase and acid protease production by solid state fermentation using *Aspergillus niger* from mangrove swamp. *Indian Journal of Fisheries* 57(1) 45-51.

Kumar A and Duhan JS (2011). Production and characterization of amylase enzyme isolated from *Aspergillus niger* MTCC-104 employing solid state fermentation. *International Journal of Pharma and Bio Sciences* **2**(3) 250-258.

Miranda OA, Salgueiro AA, Pimental NCB, Limafilho JJ, Melo EHM and Duran N (1999). Lipase production by a Brazilian strain of *Penicillium citrinium* using industrial residue. *Bioresource Technology* **69**(2) 145-149.

Norouzan D, Akbarzadeh A, Scharer JM and Young MM (2006). Fungal glucoamylases. *Biotechnology Advances* **24**(1) 80-85.

Pandey A, Webb C, Soccol CR, Larroche C (2005). *Enzyme Technology* (Asiatech publishers, Inc.) New Delhi 197-215.

Pandey A, Benjamin S, Soccol CR, Nigam P, Krieger N and Soccol VT (1999). The realm of microbial lipases in biotechnology. *Biotechnology and Applied Biochemistry* **29**(2) 119-131.

Pandey A and Soccol CR (1998). Bioconversion of biomass: a case study of lingo-cellulosics bioconversions in solid-state fermentation. *Brazilian Archives of Biology and Technology* **41**(4) 379-390.

Pandey A and Soccol CR (2000a). Economic utilization of crop residues for value addition - a futuristic approach. *Journal of Scientific and Industrial Research* **59**(1) 12-22.

Pandey A, Soccol CR and Mitchell D (2000b). New developments in solid state fermentation, I: Bioprocesses and products. *Process Biochemistry* **35**(10) 153-1169.

Pandey A, Soccol CR, Nigam P, Brand D and Mohan R (2000c). Biotechnological potential of coffee pulp and coffee husk for bioprocesses. *Biochemical Engineering Journal* **6**(2) 153-162.

Pandey A, Soccol CR, Nigam P and Soccol VT (2000d). Biotechnological potential of agro-industrial residues, I: Sugarcane bagasse. *Bioresource Technology* **74**(1) 69-80.

Pandey A, Soccol CR, Nigam P, Soccol VT, Vandenberghe LPS and Mohan R (2000e). Biotechnological potential of agro-industrial residues, II: Cassava bagasse. *Bioresource Technology* 74(1) 81-87.

Reddy AS, Jharat R and Byrne N (2003). Purification and properties of amylase from a review. *Journal of Food Biochemistry* **210**(1) 281-302.

Appendix 1
Standard Graph of Maltose
Concentration of working standard: 3mg/ml

Reagents	Blank	S1	S2	S3	S4	S5
Volume of working Standard (ml)	_	0.2	0.4	0.6	0.8	1.0
Conc. in moles	0	1.66 x10^{-9}	3.33x 10^{-9}	4.99x 10^{-9}	6.66x 10^{-9}	8.32x 10^{-9}
Distilled Water (ml)	1	0.8	0.6	0.4	0.2	0
DNS Reagent (ml)	3	3	3	3	3	3
		Incubation: Boiling water bath, 5 minutes				
Distilled Water (ml)	2	2	2	2	2	2
OD AT 520nm	0	0.12	0.24	0.36	0.48	0.60

Appendix 2