ISSN 2250-3153

Solid State Fermentation of Aspergillus niger MENA1E and Rhizopus MENACO11A for Glucoamylase Production on Agricultural residues.

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Abstract- Glucoamylases are important enzymes that allow the hydrolysis of starch and related polymers to glucose. They can be obtained from microbial as well as other sources. Studies were conducted with two fungal isolates, *Aspergillus niger* MENA1E and *Rhizopus* MENACO11A obtained locally from infected plant materials to assess their potential in producing glucoamylase. The organisms were grown on four substrates: wheat bran, rice bran, groundnut pod and maize bran. Glucoamylase was produced by both organisms on all four substrates. The highest glucoamylase activities of 2.0 U and 1.99 U respectively for *Aspergillus niger* MENA1E and *Rhizopus* MENACO11A were recorded after 48 hours on wheat bran. Significant activities of the enzyme were also produced on the other wastes studied in this report.

Index Terms- Agricultural residues, *Aspergillus,* Glucoamylase, *Rhizopus,* Solid state fermentation.

I. INTRODUCTION

The amylase family of enzymes have been well characterized through the study of various microorganisms (Ellaiah *et al*., 2002). Two major groups, endo- and exo-amylases, have been identified and are among the enzymes most studied (Horvathva *et al*., 2001; Aiyer, 2005; Anto *et al*., 2006). These enzymes represent about 25-33% of the world enzyme market, second after proteases (Cereia *et al*., 2006). Amylases are important enzymes employed in starch processing industries for hydrolysis of polysaccharides into simple sugars (Suganthi *et al*., 2011; Pandey *et al*., 1996).

Glucoamylase is an economically important enzyme because of its capacity to hydrolyse starch and related polymers into β-D-glucose as the sole end product (Valent *et al*., 1992). The principal industrial use of glucoamylase is therefore, the production of glucose, which in turn serves as a feedstock for biological fermentations in the production of ethanol or high fructose syrups (Saha and Zeikus, 1989; Pavezzi *et al*., 2008; Zambare, 2010). Glucoamylase is also used to improve barley mash for beer production (Pavezzi *et al*., 2008). It is a key enzyme in the production of sake and soy sauce (Zambare, 2010). Glucoamylase also has applications in confectionery, baking and pharmaceutical industries (Rose, 1980; Pandey *et al*., 2000). The enzyme has applications in the textile and paper industries (Pandey *et al*., 1996).

Readily available sources of glucoamylase are required for the development of economically sound fermentation industries that utilize starch as a raw material. Imported glucoamylase is the main source of this enzyme for several small scale fermentation industries in developing countries. Sustaining or increasing production levels of these small scale industries depend on improving the availability of important enzymes, principally glucoamylase, produced locally at affordable prices. Microorganisms are used to produce large quantities of industrially important enzymes including glucoamylase using cheap agricultural residues (Zambare, 2010).

Aspergillus niger and *Rhizopus sp.* have been used to produce glucoamylase for industrial activities (Hata *et al*., 1997; Fujio and Morita, 1996; Fogarty and Kelly, 1980; Selvakumar *et al*., 1994; Pandey *et al*., 1993; Pandey and Ashok, 1991; Bhatti *et al.*, 2007).

Local small and medium scale fermentation industries depend on starch-rich staple foods such as maize, rice, millet and sorghum as sources of amylases for starch hydrolysis (Ocloo and Ayernor, 2010). The use of staple food for enzyme production on large scale has the potential of increasing prices of staple foods and negatively impacting food security. A number of locally available agricultural wastes or residues could be exploited as substrates for the production of glucoamylase by microorganisms.

This study reports on the production of glucoamylase using locally isolated fungal species, *Aspergillus niger* MENA1E and *Rhizopus* MENACO11A on agro-residues using solid-state fermentation.

II. METHODOLOGY

Sources of Fungal Isolates for the Studies

Two fungal species, *Aspergillus niger* MENA1E and *Rhizopus* MENACO11A were isolated from infected plant materials and maintained pure on potato dextrose agar (PDA). The fungal isolates were stored at 4°C in a refrigerator and regenerated on freshly prepared PDA medium whenever required for experiments.

Preparation of substrates for inoculation

Wheat bran, rice bran, groundnut pods and maize bran were purchased or obtained from agro-processing mills for this study. The five substrates were separately dried and milled using a Wiley mill to 0.5 mm particle sizes (Paulchamy, 2008). Ten (10) grams of each substrate were mixed with 10 ml of Aspergillus

Complete Medium (ACM) in a 250 mL Erlenmeyer flask. ACM comprises: $(NH_4)_2SO_4$: 5.0; $Na_2HPO_4 \cdot 12H_2O$: 3.8; KH_2PO_4 3.5; $MgSO_4$ 0.5 and yeast extract 0.1 at pH 5.0 (Zaldivar-Aguero *et al*., 1997). The ACM provided the needed mineral salts required for effective growth of *Aspergillus niger* MENA1E. Similarly, ten (10) grams of each substrate for growing the *Rhizopus* MENACO11A were mixed with a mineral solution containing (g/L): $(NH_4)_2SO_4$ 20.0; K_2HPO_4 3.0; NaCl 5.0; $MgSO_4$ 5.0; Na_2HPO_4 2.0 and $CaCl_2$ 1.0 at pH 4.5 (Rauf *et al*., 2010). The mixtures of substrates and mineral solutions were autoclaved at a temperature of 121°C for 25 minutes before they were inoculated with the appropriate organism.

Inoculation of Substrate

Spore suspensions of each of the two fungal species were produced from six day old cultures grown on PDA. The spore suspension was produced by adding 12 ml of sterile distilled water (SDW) to actively growing six-day old cultures of the appropriate fungal organism. The resulting spore suspension was filtered through four layers of sterilized cheese cloth. The concentration of spore suspension was adjusted to 1×10^7 using a haemocytometer. 10 g of each substrate mixture was inoculated with 3 mL of the adjusted spore suspension in a 250 ml Erlenmeyer flask. The cultures were incubated and maintained at 30°C**.**

Assaying for Glucoamylase Activity

Inoculated substrate-mineral solution mixtures of the two fungal species were harvested at 24 hour intervals over five days (120 hours). At harvest, 10 ml of 0.021M citric acid buffer (pH, 4.6) was added to the contents of each flask. The flask was then actively shaken to ensure proper mixing of the culture mixture and the buffer. The resultant mixture from each flask was squeezed through four layers of cheese cloth. The culture filtrate from each flask was centrifuged at 7000 rpm for 15 minutes. The collected supernatant (crude enzyme) was assayed for glucoamylase activity following methods described by (Zambare, 2010 and Miller *et al*., 1959). Glucoamylase activity in each supernatant (crude enzyme) was determined by incubating at 28 °C for 10 min, a reaction mixture containing 1.0 ml of 50 mM citrate buffer (pH 4.6), 1.0 ml starch solution (1 %, w/v) and 1.0 ml of the crude enzyme. The released reducing sugar in the reactive mixture was measured using 3, 5-dinitrosalicyclic acid (DNSA) reagent (Miller *et al*. 1959) using glucose as a standard. Glucoamylase activity unit (U) was expressed as the amount of enzyme releasing one µmole of glucose equivalent per minute under assay condition.

III. RESULTS AND DISCUSSION

The two fungal species, *Aspergillus niger* MENA1E and *Rhizopus* MENACO11A investigated in this study produced glucoamylase on all four agro residues tested. Using glucoamylase produced from *Aspergillus niger* MENA1E, wheat bran gave the highest glucoamylase activity (2 U) at 48 hours of incubation followed by rice bran (1.58 U) at 96 hours of incubation in Table 1.

Table 1: Glucoamylase production from *Aspergillus niger* MENA1E

	Substrate			
Time (Hours)	Wheat bran	Rice bran	Groundnut pod	Maize bran
	Glucoamylase enzyme production(U)			
24	1.50±0.1	1.01 ±0.2	0.44±0.1	0.14±0.1
48	2.00±0.1	1.21 ±0.1	0.83 ±0.1	0.28±0.1
72	1.56 ±0.1	1.52 ±0.1	1.11±0.2	0.38±0.2
96	1.66±0.1	1.58 ±0.1	1.39±0.1	0.22±0.1
120	1.79±0.2	0.92 ±0.1	1.17±0.2	0.17±0.2

For glucoamylase produced from *Rhizopus* MENACO11A, wheat bran also gave the highest glucoamylase activity (1.99 U) at 48 hours of incubation followed by rice bran (1.55 U) at 72 hours of incubation in Table 2.

Table 2: Glucoamylase production from *Rhizopus* MENACO11A

	Substrate			
Time (Hours)	Wheat bran	Rice bran	Groundnut pod	Maize bran
	Glucoamylase enzyme production(U)			
24	1.47±0.1	0.89 ±0.1	0.42±0.2	0.13±0.1
48	1.99±0.1	1.11 ±0.1	0.8 ±0.1	0.17±0.1
72	1.5 ±0.1	1.55 ±0.1	1.33±0.1	0.15±0.2
96	1.55±0.1	1.5 ±0.1	1.11±0.1	0.21±0.1
120	1.55±0.1	0.94 ±0.1	0.83±0.2	0.11±0.1

Wheat bran has been reported by several workers as a very suitable substrate for the production of glucoamylase (Pandey *et al*., 1999; Anto *et al.,* 2006; Kaur *et al.,* 2003). The current results suggest rice bran as a possible substitute for wheat bran among the substrates considered. Similar results were obtained by Zambare (2010).

In Table 1, the activity of glucoamylase from *Aspergillus niger* MENA1E on each substrate increased to a maximum and then declined with time. This observation may be ascribed to utilisation of minerals in the fungal culture by the fungal species (Hema Anto *et al*., 2006). A similar trend was observed for *Rhizopus sp*. MENACO11A (Table 2) except on maize bran where activities of glucoamylase remained fairly constant. Glucoamylase from *Aspergillus niger* and *Rhizopus sp*. are reported to be structurally different and this may be responsible for the different activities observed. The carbohydrate chains (containing mannose and glucosamine in the N-acetyl form) of the *Rhizopus sp.* enzyme are linked by O-glycosidic and N-glycosidic linkages to the protein, while carbohydrate chains (containing mannose, glucose, and galactose) of the *Aspergillus niger* enzyme are linked by O-glycosidic linkages only to the protein (Pazur *et al*., 2010).

During solid-state fermentation, higher moisture level decreases porosity, changes substrate particle structure, promotes development of stickiness and lowers oxygen transfer, whereas lower moisture content causes reduction in the solubility of nutrients of the solid substrate, lower degree of swelling and higher water tension (Hema Anto *et al*., 2006). The moisture content was maintained at a fixed value in this study, which might not necessarily be optimal for the two fungal species.

Analysis of variance conducted on the data using Statistix 9 statistical software showed *Aspergillus niger* MENA1E, wheat bran and 48 hours of incubation as the best combination for maximum glucoamylase production (Tables 3.1, 3.2 and 3.3).

Table 3 Main effect for organisms

Organism	GA
Rhizopus sp.	0.797b
Aspergillus niger	0.859a
F.Pr	**<0.001**
LSD(0.05)	**0.022**
%CV	**10.2**

Table 3.1 Main effect for media

Medium	GA
Wheat bran	1.315a
Rice bran	0.972b
Groundnut pod	0.774c
Maize bran	0.179d
F.Pr	**<0.001**
LSD(0.05)	**0.025**
%CV	**10.2**

Table 3.2 Main effect for incubation period

Time (hours)	GA
24	0.499d
48	0.752a
72	0.757a
96	0.703b
120	0.630c
F.Pr	**<0.001**
LSD(0.05)	**0.024**
%CV	**10.2**

The mean values in each column followed by the same letter are not significantly di**f**ferent

Enzyme production by microorganisms is affected by a number of factors including temperature, substrate particle size, pH and nitrogen concentration (Pandey *et al*., 1994). Improving glucoamylase production by microbial organisms through exploitation of these factors has been reported by several workers (Irfan *et al*., 2012). The optimization of these factors is suggested by these initial results for maximum production of glucoamylase on readily available substrates by *Aspergillus niger* MENA1E and *Rhizopus sp.* MENACO11A.

IV. CONCLUSIONS

The two fungal isolates, *Aspergillus niger* MENA1E and *Rhizopus* MENACO11A isolated locally from infected plant materials were capable of producing the enzyme glucoamylase on four substrates, wheat bran, rice bran, groundnut pod and maize bran. Individually, *Aspergillus niger* MENA1E was found to produce higher glucoamylase activity than *Rhizopus* MENACO11A. With respect to media, wheat bran was most suitable for producing maximum glucoamylase activity. Generally, the most favourable period of incubation was 48 hours. The study suggests that native *Aspergillus niger* MENA1E and *Rhizopus* MENACO11A could be used to produce high glucoamylase activity at optimized conditions for industrial purposes.

REFERENCES

[1] Abdul Rauf, Muhammad Irfan, Muhammad Nadeem, Ishtiaq Ahmed and Hafiz Muhammad Nasir Iqbal (2010) Optimization of Growth Conditions for Acidic Protease Production from Rhizopus oligosporus through Solid State Fermentation of Sunflower meal. Word Academy of Science, Engineering and Technology, 48: 608 – 609.

[2] Aiyer, P.V.; Arts, S.P.T. and College, S. (2005) Amylases and their Applications. African Journal of Biotechnology, 4, 1525–1529.

[3] Anto, H.; Trivedi, U.P. and Patel, K.C. (2006) Glucoamylase Production by Solid-State Fermentation using Rice Flake Manufacturing Waste Products as Substrate. Bioresource Technology, 97, 1161-1166.

[4] Banks, G.T.; Binns, F. and Cutcliffe, R.L. (1976) Recent Development in the Production and Industrial Applications of Amylolytic Enzymes Derived from Filamentous Fungi. In: Progress Industrial Microbiology, 6, 95-139.

[5] Bhatti, H.N.; Rashid, M.H. and Nawaz, R. (2007) Optimization of Media for Enhanced Glucoamylase Production in Solid-State Fermentation by Fusarium solani. Food Technology and Biotechnology, 45(1), 51–56.

[6] Cereia, M.; Guimarães, L.H.S.; Peixoto-Nogueira, S.C.; Jorge, J.A.; Terenzi, H.F.; Greene, L.J. and Polizeli, M.D.L.T.M. (2006) Glucoamylase Isoform (GAII) Purified from a Thermophilic Fungus Scytalidium thermophilum 15.8 with Biotechnological Potential. African Journal of Biotechnology, 5, 1239–1245.

[7] Ellaiah, P.; Adinarayana, K.; Bhavani, Y.; Padmaja, P. and Srinivasulu, B. (2002) Optimization of Process Parameters for Glucoamylase Production Under Solid State Fermentation by a Newly Isolated Aspergillus species.

Process Biochemistry, 38(4), 615–620.

[8] Pavezzi, F.C.; Eleni Gomes, E. and da Silva, R. (2008) Production and characterization of glucoamylase from fungus Aspergillus awamori expressed in yeast saccharomyces cerevisiae using different carbon sources. Brazilian Journal of Microbiology, 39, 108-114.

[9] Fogarty, W.M. and Kelly, C.T. (1980) Amylases, Amyloglucosidases and Related Glucanases. In: Microbial Enzymes and Bioconversions

[10] (Ed. Rose, A. H.,). London, Academic Press, pp. 115-170.

[11] Fujio, Y. and Morita, H. (1996) Improved Glucoamylase Production by Rhizopus species A-l 1 Using Metal-Ion Supplemented Liquid Medium. Journal of Fermentation Bioengineering, 82(6), 554–557.

[12] Hata, Y.; Ishida, H.; Kojima, Y.; Ichikawa, E.K.A.; Suginami, K. and Imayasu, S. (1997) Comparison of Two Glucoamylases Produced by Aspergillus oryzae in Solid-State Culture and in Submerged Culture. Journal of Fermentation and Bioengineering, 84, 532-537.

[13] Horváthová, V.; Janeček, Š. and Šturdík, E. (2001) Amylolytic Enzymes: Molecular Aspects of Their Properties. General Physiology and Biophysics, 20, 7–32.

[14] Irfan, M.; Muhammad, N. and Quratualain, S. (2012) Media Optimization for Amylase Production in Solid State Fermentation of Wheat Bran by Fungal Strains. Journal of Cell and Molecular Biology, 10(1): 55-64.

[15] Kaur, P.; Grewal, H.S. and Kocher, G.S. (2003) Production of α-amylase by Aspergillus niger using Wheat Bran in Submerged and Solid State Fermentations. Indian Journal of Microbiology, 43, 143–145.

[16] Miller Gail Lorenz (1959) Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar. Analytical Chemistry, 31 (3): 426–428.

[17] Ocloo, F.C.K. and Ayernor, G. S. (2010) Production of Alcohol from Cassava Flour Hydrolysate. Journal of Brewing and Distilling, 1(2): 15–21.

[18] Pazur, J.H.; Liu, B.L. and Miskiel, F.J. (2010) Comparison of the Properties of Glucoamylases from Rhizopus niveus and Aspergillus niger. Biotechnology and Applied Biochemistry, 12, 63–78.

[19] Pandey, A.; Nigam, P.; Soccol, C.; Soccol, V.; Singh, D.; Mohan, R. (2000) Advances in Microbial Amylases. Biotechnology and Applied Biochemistry, 31, 135–152.

[20] Pandey, A. and Radhakrishna, S. (1993) The Production of Glucoamylase by Aspergillus niger NCIM 1245. Process Biochemistry, 28(3), 305-9.

[21] Pandey, A.; Selvakumar, P. and Ashakumary, L. (1996). Performance of a Column Bioreactor for Glucoamylase Synthesis by Aspergillus niger in Solid State Fermentation. Process Biochemistry, 3(1), 43–46.

[22] Pandey, A.; Selvakumar, P. and Ashakumary, L. (1994) Glucoamylase Production by Aspergillus niger on Rice Bran is Improved by Adding Nitrogen Sources. World Journal of Microbiology and Biotechnology, 10 (3): 348-349.

[23] Pandey, A.; Selvakumar, P.; Soccol, C.R. and Nigam, P. (1999) Solid State Fermentation for the Production of Industrial Enzymes. Current Science, 77(1), 149 - 62.

[24] Pandey, A.; Selvakumar, P.; Soccol, R. and Nigam, P. (1991) Solid State Fermentation for the Production of Industrial Enzymes. Bioresearch Technology, 37, 169–172.

[25] Paulchamy, C. (2008) Solid-State Cultivation of Aspergillus niger NCIM 548 for Glucoamylase Production on Groundnut Shell. Journal of Microbiology, 5, 1-6.

[26] Pavezzi, F.C.; Gomes, E. and Silva, R. (2008) Production and Characterization of Glucoamylase from Fungus Aspergillus awamori Expressed in Yeast Saccharomyces Cerevisiae using Different Carbon Sources. Brazilian Journal of Microbiology, 39, 108-114.

[27] Rose, H.A. (1980) Microbial Enzymes and Bioconversions. Economic Microbiology, 5, 116–117.

[28] Saha, B.C. and Zeikus, J.G. (1989) Microbial Glucoamylases. Brazilian Journal of Chemical Engineering, 41(2): 57–64.

[29] Selvakumar, A.L.P. and Pandey, A. (1994) Solid State Fermentation. (Ed. Pandey, A.), Wiley Eastern Publishers, New Delhi, pp. 33–37.

[30] Shivaramakrishnam, S.; Ganghadharan, D.; Nampoothiri, K.M.; Soccol, C.R. and Pandey, A. (2007) Alpha Amylase Production by Aspergillus oryzae Employing Solid-State Fermentation. Journal of Scientific and Industrial Research, 66, 621-626.

[31] Singh, H. and Soni, S.K. (2001) Production of Starch-Gel Digesting Amyloglucosidase by Aspergillus oryzae HS-3 in Solid State Fermentation. Process Biochemistry, 37, 453–459.

[32] Suganthi, R.; Benazir, J.F.; Santhi, R.; Ramesh K.V.; Anjana Hari Nitya, A.H.; Meenakshi, N.K. A.; Kavitha, G. and Lakshmi, R. (2011) Amylase Production by Aspergillus niger Under Solid State Fermentation using Agro Industrial Wastes. International Journal of Engineering Science and Technology, 3, 2 - 3.

[33] Zaldivar-Aguero, J.M.; Badino, A.C.; Vilaça, P.R.; Facciotti, M.C.R. and Schmidell, W. (1997) Influence of phosphate concentrations on glucoamylase production by Aspergillus awamori in submerged culture. Brazilian Journal of Chemical Engineering, 14, 1-4

[34] Zambare, V. (2010) Solid State Fermentation of Aspergillus oryzae for Glucoamylase Production on Agro residues. International Journal of Life Science, 4:16-25.

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