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Glucoamylase Production by *Aspergillus niger* in Solid State Fermentation with Paddy Husk as Support

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Aspergillus niger CFTRI 1105 produced the highest amount of glucoamylase 875.5 Ug^{-1} DS (3rd day) in a medium containing soy meal powder (200.0 g), paddy husk (460.0 g), mineral solution (20.0 ml) and tap water (180.0 ml). Glucoamylase activity obtained in media containing beef and soy meal powder having same elemental nitrogen level and starch concentration were closer to each other. Optimization of moisture content and spore inoculum size improved glucoamylase production with reduction in production time. Optimized amount of paddy husk to soy meal powder ratio was 7.0:3.0. By optimizing the conditions, production of glucoamylase increased to 1853.7 Ug^{-1}DS on the 2nd day.

Keywords : Solid state fermentation, Paddy husk, Soy meal powder, Glucoamylase, Dry substrate.

Glucoamylase is produced by submerged (Jansz et al. 1977; Ramadas et al. 1996; Kolhekar et al. 1985) and solid state (Oriol et al. 1988; Ramadas et al. 1996; Ghildyal et al. 1985) fermentation (SSF) processes. Solid state fermentation technique is the traditional fermentation method of South-East Asia (Aidoo et al. 1982; Cannel and Moo-Young 1980 a, b; Hasseltine 1977 a,b and is still in use (Ghildyal et al. 1992; Gowthaman et al. 1993) for the production of α-amylase (Ramesh et al. 1993), glucoamylase (Ghildyal et al. 1985), protease (Battaglino et al. 1991: Shanmuganathan et al. 1993) gibberellic acid (Kumar and Lonsane 1989), fibrinolytic enzyme (Tao et al. 1997) and pectinase Ghildyal et al. 1981). Previous reports suggested that wheat bran (Ghildyal et al. 1985; Ramakrishna et al. 1982; Ramadas, et al. 1996) with additional nutrients such as corn meal and minerals was useful for the production of glucoamylase and other enzymes by SSF processes. Wheat bran is not available in Sri Lanka and hence in the present study, rice bran and paddy husk as alternative substrates were used (Pandey et al. 1994 a;b) and / or support (Arasaratnam et al. 1997) for glucoamylase production. Since the use of paddy husk was attractive as a support, it was decided to optimize the culture conditions and formulate a medium for *Aspergillus niger* CFTRI 1105.

Materials and Methods

Materials : Soy and corn flours were prepared by milling soy seeds and corn grains (*Zea mays.* L) purchased from local market. Paddy husk available in the local grinding mill was used. Waxy maize starch was from Stardex, Sweden. Soy meal (Delmage, Colombo) was ground to powder in a domestic grinder. Beef and ox intestines were purchased from a local slaughter house and minced in a domestic mincer.

Microorganism : Aspergillus niger CFTRI 1105 was from CFTRI, Mysore, India. The organism was sub-cultured once in every two weeks on Potato Dextrose Agar (PAD) slants and stored at 4°C (Navaratnam et al. 1996). Whenever the spores were required, they were suspended in 1.0% (v/v) Tween -80 and the number of spores were counted using a haemocytometer.

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Glucoamylase activity : Enzyme activity was determined as described previously (Arasaratnam, et al. 1997) and the activity of glucoamylase is presented as U g^{-1} DS (dry substrate).

Comparison of corn flour, soy flour and soy meal powder: Paddy husk (460.0 g) was mixed with corn flour (medium I) or soy flour (medium II) or soy meal powder (medium III), mineral solution (containing (g^{-1}) $CuSO_4$. $5H_2O$, 0.7 g; $FeSO_4$. $7H_2O$, 0.7 g and $ZnSO_4$. $7H_2O$ in 2N HCl) and tap water (Table 1) in 5 litre conical flasks and sterilized. The pH of the medium was 4.0. The spore suspension (4.5 X 10^7 ml^{-1}, 20.0 ml, in 1.0%, v/v Tween-80) inoculum was added and incubated at 30°C up to 4 days. Enzyme activity and moisture content (Pearson 1976) of the media were measured.

Effect of soy meal powder concentration : Composition of the media prepared with different concentrations of soy

TABLE 1. COMPOSITIONS OF THE MEDIA TO STUDY THE EFFECTS OF CORN FLOUR, SOY FLOUR AND SOY MEAL POWDER

	Medium		
Constituents	I	II	III
Corn flour, g	200.0	-	-
Soy flour, g	-	200.0	-
Soy meal powder, g	-	-	200.0
Paddy husk, *g	460.0	460.0	460.0
Mineral solution **ml	20.0	20.0	20.0
Tap water, ml	180.0	180.0	180.0
Wet weight of substrate, g	200.0	200.0	200.0
Dry weight of substrate, g	176.0	180.0	180.0
Total activity, U	57200.00	122958.0	157590.0
Activity, U g^{-1} substrate	325.0	683.1	875.5
N/C ratio	0.12	0.58	1.57

The media were taken in 5 litre conical flasks

*Paddy husk (460 g) was soaked in water and suction-dried before mixing

**Mineral solution contained (g^{-1}) $CuSO_4$. $5H_2O$, 0.7: $FeSO_4$. $7H_2O$, 0.7 and $ZnSO_4$. $7H_2O$, 0.7 dissolved in 2M HCL

The ratio between dry paddy husk and wet substrate was 7.0:3.0