Paddy husk support and rice bran for the production of glucoamylase by *Aspergillus niger*

Vasanthy Arasaratnam*, Ketheeswary Mylvaganam & Kandiah Balasubramaniam

Department of Biochemistry, Faculty of Medicine, University of Jaffna, Kokuvil, Sri Lanka

Summary *Aspergillus niger* CFTRI 1105 was cultivated on solid medium for glucoamylase production. Glucoamylase activity obtained was 83.7 U g^{-1} DFR (Dry Fermentation Residue) in a medium containing rice bran (100 g), corn flour (2 g), stock mineral solution (10 mL) and tap water (90 mL). When corn flour (2 g) in the medium was substituted with soya flour (2 g) no significant increase in glucoamylase was observed. The effects of soya flour, urea and peptone at the same elemental nitrogen concentration as with corn flour as carbon source on glucoamylase production were investigated. Supplementation with soya flour gave the highest glucoamylase activity (121 U g^{-1} DFR) at 72 h and addition of paddy husk to a medium containing corn and soya flour altered the enzyme production from 121 U g^{-1} DFR to 71.3 U g^{-1} DFR. Addition of gingili oil and coconut oil to the medium caused no improvement in glucoamylase production.

Keywords Corn flour, dry fermentation residue, solid state fermentation, soya flour.

Introduction

α-Amylase and glucoamylase are the two key enzymes used in the liquefaction and saccharification of starch (Arasaratnam & Balasubramaniam, 1993). Glucoamylase can be produced by submerged (Jansz *et al.*, 1977) and solid state (Oriol *et al.*, 1988) fermentation (SSF) processes; the latter were the traditional fermentation methods of South-East Asia (Cannel & Moo-Young, 1980; Battaglino *et al.*, 1991; Hasseletine, 1977) and are still in use (Ghildyal *et al.*, 1992; Gowthaman *et al.*, 1993) for the production of alpha amylase (Ramesh *et al.*, 1993), glucoamylase (Ghildyal *et al.*, 1985), protease (Battaglino *et al.*, 1991), giberellic acid (Kumar & Lonsane, 1989) and pectinase (Ghildyal *et al.*, 1981). Previous reports (Ghildyal *et al.*, 1985; Ramakrishna *et al.*, 1982) suggest that wheat bran with additional nutrients such as corn meal and minerals is a useful medium for the production of glucoamylase and other enzymes in SSF processes. Wheat bran is not freely available in Sri Lanka and hence we have tried to use rice bran and paddy husk as alternative substrates and/or support for glucoamylase production. Since the use of rice bran or paddy husk has not been as extensively studied as wheat bran, we have undertaken this preliminary feasibility study. Rice bran is used to feed animals and birds while the paddy husk is usually burnt and some of the ash is added to the paddy field. Most of the paddy husk is wasted. Thus if paddy husk could be used in SSF its wastage could be avoided by using the fermented residue as poultry feed or fertilizer. The established glucoamylase production in SSF process by *Aspergillus niger* CFTRI 1105 was taken as the basis for the use of rice bran and/or paddy husk. The coconut (*Cocos nucifera* L.) and gingili (Sesame; *Sesamum indicum* L.) oils were included in the medium because they are rich in saturated fatty acids (Fritz & Johnson, 1988) such as lauric acid (45–50%) and myristic

*Correspondent: c/o Mrs A. Balasubramaniam, 32/3 Railway Avenue, Colombo 5, Sri Lanka.

(18–20%) acids (iodine number is 8), and in unsaturated fatty acids (Dutta & Ghosh, 1972) such as oleic (40%) and linoleic (39%) acids (iodine number is 114, Jurriens, 1968), respectively (Wickramanayake, 1987), and may show variations in membrane permeability for extracellular glucoamylase.

Materials and methods

Soya flour and corn flour were prepared by milling soya seeds and corn grains (*Zea mays* L.) purchased from local market. Locally available paddy husk, rice bran, gingili oil, coconut oil and urea were used. Peptone was from Oxoid, England. Wheat bran was a gift from Central Food Technological Research Institute (CFTRI), Mysore, India. Waxy maize starch was donated by Stadex, Sweden.

Microorganism

Aspergillus niger CFTRI 1105 was from CFTRI, Mysore, India. The organism was subcultured once every two weeks on Potato Dextrose Agar (PDA) slants and stored at 4 °C (Navaratnam *et al.*, 1996). Whenever the spores were required, they were suspended in 1% (v/v) Tween-80 and the number of spores were counted using a haemocytometer.

Glucoamylase activity

Enzyme was extracted by mixing 1 g of dried mouldy medium with distilled water (9 mL) for 30 min and centrifuging (6000 g, 10 min). Supernatant was appropriately diluted with acetate buffer (pH 4.0) to a final concentration to 0.02 M and 0.5 mL of the diluted enzyme was preincubated at 60 °C for 30 min, mixed with 0.5 mL of 20 g L^{-1} starch in 0.02 M acetate buffer (pH 4.0) and incubated at 60 °C for 10 min. Reducing sugar produced was measured by the dinitrosalicylic acid method (Miller, 1959) where one unit (U) of glucoamylase activity is the amount of enzyme that liberates one μmole of glucose in one minute. The activity of glucoamylase is presented as U g^{-1} DFR (Dry Fermentation Residue). All data are means of at least two samples which agreed within 5%.

Comparison of the effects of corn flour and soya flour on glucoamylase production

Rice bran (100 g) was mixed either with corn flour (Medium I) or soya flour (Medium II), mineral solution and tap water (Table 1) in a 1 litre conical flask and sterilized. The spore suspension (4.5×10^7 mL^{-1}, 20 mL, in 1% (v/v) Tween-80) inoculum was added and incubated at 30 °C for up to 6 days. Enzyme activity and moisture content (Pearson, 1976) of the media were measured.

Comparison of glucoamylase production in media containing rice bran and wheat bran

To either rice bran (100 g; Medium I, Table 1) or wheat bran (100 g, Medium III, Table 1), corn flour, mineral solution and tap water were added as described above and glucoamylase production was monitored.

Effect of different nitrogen sources on glucoamylase production

Different nitrogen sources such as soya flour, urea or peptone were mixed with corn flour and mineral solution and tap water (Table 1). To the media containing urea and peptone, waxy maize starch was added to equalize the starch content in the media. Control medium (Medium VII, Table 1) contained corn flour, waxy maize starch, mineral solution and tap water. Spores of *Aspergillus niger* were inoculated and the experiment was conducted as above.

Effect of paddy husk on glucoamylase production

Corn flour, soya flour, paddy husk, mineral solution and tap water were mixed together (Medium VIII, Table 1). A control medium was prepared by omitting paddy husk (Medium IV, Table 1). Glucoamylase production by *A. niger* was monitored.

Effect of gingili oil and coconut oil on glucoamylase production

To the Medium VIII (Table 1) either gingili oil (0.2%, v/w) or coconut oil (0.2%, v/w), or 0.1%

Table 1 Compositions of the media prepared to study the effects of corn flour and soya flour, rice bran and wheat bran, different nitrogen sources, paddy husk and different amounts of gingili oil and coconut oil on glucomylase production by *Aspergillus niger* CFTRI 1105 by solid state fermentation at pH 4.0 and 30 °C

	Medium											
Constituents	**I**	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**X**	**XI**	**XII**
Rice bran (g)	100	100	—	—	—	—	—	—	—	—	—	—
Wheat bran (g)	—	—	100	—	—	—	—	—	—	—	—	—
Corn flour (g)	2	—	2	100	100	100	100	100	100	100	100	100
Soya flour (g)	—	2	—	100	—	—	—	100	100	100	100	100
Urea (g)	—	—	—	—	13.7	—	—	—	—	—	—	—
Peptone (g)	—	—	—	—	—	44.8	—	—	—	—	—	—
Waxy maize starch (g)	—	—	—	—	51.1	51.1	51.1	—	—	—	—	—
Paddy husk*** (g)	—	—	—	—	—	—	—	460	460	460	460	460
Gingili oil (ml)	—	—	—	—	—	—	—	—	1.72	—	0.86	1.72
Mineral solution** (mL)	10	10	10	20	20	20	20	20	20	20	20	20
Tap water (mL)	90	90	90	180	180	180	180	180	180	180	180	180
Glucoamylase activity												
At hours	96	96	72	72	72	96	72	72	96	96	96	96
U g^{-1} DFR@	83.7	86.5	672.1	121.0	5.8	61.4	70.9	71.3	38.0	28.6	27.4	18.6
U mL^{-1}	14.3	18.0	121.6	16.2	0.8	7.5	9.4	9.5	5.2	4.5	4.2	2.9
Moisture content of DFR(%)	14.6	12.5	13.2	39.8	38.3	57.5	40.1	40.2	28.5	22.0	26.6	21.1

*Media I, II and III were taken in 1-litre, IV, V, VI and VII in 2-litre and VIII, IX, X, XI and XII 5-litre conical flasks.

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

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

@Dry fermentation residue (1 g) was mixed with 9.0 mL of distilled water. Enzyme was appropriately diluted with acetate buffer, pH 4.5 and glucoamylase activity was determined. One (U) is that which produces 1μmole glucose in one minute at 60 °C.

(v/w) each of gingili oil and coconut oil or 0.2% (v/w) each of gingili oil and coconut oil were added (Table 1). Sterilized media were inoculated with spores and the glucoamylase production was monitored.

Results and discussions

Comparison of the effects of corn flour and soya flour on glucoamylase production

Glucoamylase produced in rice bran containing either corn flour or soya flour was almost the same. In both conditions maximum activity was obtained at 96 h and the activities in Media I and II were 83.7 and 86.5 U g^{-1} DFR, respectively (Table 1). The activity exceeded 60 U g^{-1} DFR at 72 and 120 h. *Bacillus licheniformis* 6346 produced more α-amylase in media containing rice flour or soya flour and rice husk than in a medium containing corn flour and rice husk (Tambyrajah *et al.*, 1995). *M. dispersus* NRRL 3103, *A. elegans* NRRL 3104 and *R. oilgopous* NRRL 2710 preferred wheat bran to soya bean and wheat as solid substrate for protease production. The second preference for *M. dispersus* NRRL 3103 and *A. elegans* NRRL 3104 was soya bean while that for *R. oilgopous* NRRL 2710 was wheat (Wang *et al.*, 1974). Thus it can be observed that for different organisms to produce different types of enzymes the preference of carbon source in the solid medium varies. Because the glucoamylase produced in both the media studied was the same, it was decided to use corn flour since it is cheaper.

Comparison of glucoamylase production in media containing rice bran and wheat bran

Glucoamylase produced in the medium containing wheat bran and corn flour was higher (≈7.5 times more) than that obtained in the medium containing rice bran and corn flour (Table 1) and maximum glucoamylase was produced at 72 and 96 h days, respectively. Pandey *et al.* (1994) obtained 220 U of glucoamylase activity per gram of dry substance from *Aspergillus niger* at 96 h, when rice bran was used as substrate. When *Aspergillus niger* CISIR N4 was grown on wheat

bran to produce glucoamylase and the enzyme was extracted using medium:distilled water in the ratio of 1:9, 44 U mL^{-1} of glucoamylase activity was obtained at 96 h (Ramadas *et al.*, 1994). With the same organism in submerged fermentation Ramadas *et al.* (1994) obtained 66 U mL^{-1} glucoamylase activity at 66 h. Differences in enzyme production cannot be attributed to variations in carbohydrate and protein contents between rice and wheat brans. The protein and carbohydrate contents of wheat bran were 15.2% and 34.0%, respectively (Ramakrishna *et al.*, 1982) whereas those of rice bran were 10.3 and 34.0%, respectively (Wickramanayake, 1987). The results indicate that wheat bran is better for glucoamylase production than rice bran. However, use of wheat bran is not feasible in Sri Lanka. Hence it was decided to replace wheat bran with corn flour in the medium rather than with rice bran. Additional nutrients were added to enrich the medium to improve glucoamylase production.

Effect of different nitrogen sources on glucoamylase production

When corn flour medium was supplemented with either soya flour, urea or peptone (to give 8.0 g of elemental nitrogen in the total medium), glucoamylase production (Table 1) was highest in the medium with soya flour. Enzyme activity was better in the control (Medium VII) than in urea-supplemented medium (V) suggesting an inhibition of glucoamylase production (Table 1). In peptone-supplemented medium, maximum enzyme production occurred 24 h later (at 96 h) than in control (Medium VII) and soya flour-supplemented medium. It was expected that enzyme production would be faster in peptone-containing medium than in soya flour-supplemented medium, because the amino acids in peptone can be more easily utilized than the proteins of soya flour. In this experiment the elemental nitrogen level and carbohydrate content were kept constant. These results indicate that rather than the quantity of total nitrogen and carbohydrate, the content of other nutrients in soya flour was responsible for the increased enzyme production. Furthermore, the nutrients of corn flour alone (control, Medium VII) seem to be better for glucoamylase production than with peptone or urea supplementation. In addition waxy maize starch contained starch and no proteins (Arasaratnam, 1989). Glucoamylase production was 1.7 times more in soya flour-supplemented corn flour medium (Table 1, Medium IV) than in control (Table 1, Medium VII). Therefore soya flour was supplemented to corn flour for the following experiments. The results reported by Pandey *et al.* (1994) were contradictory to our results. They observed an increase in glucoamylase production by *Aspergillus niger* from 220 U g^{-1} dry substance to 339 U g^{-1} dry substance at 96 h when rice bran was supplemented with peptone. Senthuran *et al.* (1995) reported that medium composed of rice bran supplemented with soya flour was best among the media containing rice bran supplemented with yeast extract, peptone and intestinal proteins for protease production by *Aspergillus niger*. *Aspergillus* sp. CM_1 on surface culture produced reduced amounts of citric acid in the presence of soya flour and soya meat powder while doubling the concentration of peptone in the medium increased citric acid production from 47.1 g L^{-1} to 58.0 g L^{-1} (Navaratnam *et al.*, 1995).

Effect of paddy husk on glucoamylase production

In this experiment paddy husk was mixed with the corn flour medium in a 7.0:3.0 ratio (Battaglino *et al.*, 1991). Activity of glucoamylase obtained in different media is presented in Table 1. Glucoamylase activity obtained per gram dry substrate (DS) was two times more in the medium containing paddy husk (Medium VIII, 238.4 U g^{-1} DS) than in the control (Table 1, Medium IV, 121 U g^{-1} DS). Thus nutrients in the medium were better utilized in the presence of husk than in its absence. The distribution of paddy husk in between the medium must be aiding air circulation and mass transfer of nutrients for the organism. Thus addition of husk to the medium helps the organism to utilize the nutrients better than in control medium. The activities in the extracts show opposite values of enzyme activity. Since the nutrients were better utilized in presence of rice husk, it was incorporated into the medium for the following experiment. Glucoamylase production could be improved by optimizing the medium and culture conditions.

Effect of gingili oil and coconut oil on glucoamylase production

The results indicate that the gingili oil and coconut oil at different concentrations have delayed and decreased glucoamylase production (Table 1). Among the oils, 0.2% (v/w) coconut oil seemed to inhibit the enzyme production or secretion more than 0.2% (v/w) gingili oil. Further, the higher concentration of the mixture of oils (0.2%, v/w) decreased the enzyme production more than the lower concentration. *Bacillus licheniformis* produced an increased amount of α-amylase, when medium containing rice husk with additional nutrients was supplemented with 0.9% (v/w) gingili oil and 0.3% (v/w) coconut oil (Tambyrajha *et al.*, 1995). As gingili oil is rich in oleic and linoleic acids, it was expected that its addition would improve the membrane permeability to glucoamylase. However, both the oils rich in saturated or unsaturated fatty acids showed a reduction in enzyme production or secretion. Furthermore we can generally state that saturated fatty acids inhibited the enzyme production or secretion more than unsaturated fatty acids and under the experimental conditions, addition of the oils had no useful effect.

Conclusions

The results indicated that rice bran is not comparable to wheat bran. Soya flour is a better nitrogen source than peptone or urea in a medium containing corn flour. Incorporation of paddy husk into a medium containing corn flour and soya flour improved glucoamylase production. Even although rice bran was not as useful as wheat bran, paddy husk improved glucoamylase production by acting as a support. Addition of gingili and coconut oils did not improve enzyme production. Further investigations are in progress to increase glucoamylase production by improving the medium.

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