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Production and optimization of glucoamylase from agro waste by using *Aspergillus niger* and *Aspergillus flavus*

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ABSTRACT

Submerged fermentation of *Aspergillus niger* and *Aspergillus flavus* was carried out for enhanced production of glucoamylase using different substrates like rice bran, saw dust and mixture of rice bran and saw dust. The submerged fermentation with *Aspergillus niger* showed highest enzyme activity using rice bran as a substrate. The physical and chemical parameters were also optimized. Maximum enzyme activity (1.16±0.76 IU\ml) of rice bran was achieved under optimum growth condition such as pH 4, incubation temperature 2525℃ and substrate concentration 5gm.

Keywords: Glucoamylase, *Aspergillus niger, Aspergillus flavus,* Agrowaste, Submerged fermentation.

1. INTRODUCTION

Glucoamylase (α-1,4 glucan-glucohydrolases, EC (3.2.1.3) is exoenzymes of great importance for saccharificiation of starchy materials and other related oligosaccharides. Glucoamylase consecutively hydrolyzes 1,4-alpha-glucosidic bords from the non-reducing ends of starch and 1,6-alpha – glucosidic linkages in polysaccharides yielding glucose as the end – product, which in turn serves as a feedstock for biological fermentations [1,2]. Currently, amylases have a great importance in biotechnology with a wide spectrum of applications, such as textile industry, cellulose, leather, detergents, liquor, bread, children cereals, ethanol production, and high fructose syrups production and in various strategies in the pharmaceutical and chemial industries such as the synthesis of pure drugs and agrochemicals [3].

The screening and identification of filamentous fungi capable of secreting extracellular enzymes with biotechnological potential are activities of great importance. Glucoamylase is produced by a variety of fungi but the exclusive production of this enzyme in industry have been achieved mainly by *Aspergillus niger*, *Aspergillus oryzae* , *Aspergillus awarmori* and *Aspergillus terreus*[4,5] probably because of their ubiquitous nature and non-fastidious nutritional requirements of these organisms.

The present study is undertaken to enhance the production of glucoamylase capacity by endophytic fungus *Aspergillus* sp under submerged fermentation using agro industrial waste and optimization of the parameters, which leads to an improvement of the environmental conditions favoring the maximal exploration of fungal capacity for overproducing of glucoamylase in submerged fermentation.

2. MATERIALS AND METHODS

2.1. Microorganism

Soil and substrates sample were collected from the agricultural waste dumping area at Mannargudi, Thiruvarur District, Tamil Nadu.The fungal species were identified by wet mount technique. Among the fungal isolates to screen the glucoamylase producing fungal strain by using starch hydrolysis method. The fungal isolate was streaked on the starch agar media separately then the plates were incubation at 37℃ for 3 days. After incubation the iodine solution was poured on the plates for formation of clear zone around the colonies[6].

2.1. Glucoamlase production

The growth of *Aspergillus niger* and *Aspergillus flavus* in pre inoculum broth, 10ml was transferred to 90ml of potato dextrose broth. Then the inoculum was incubated at 28℃ for 3-5 days.A five gram of substrate of rice bran, saw dust, mixture of rice bran and saw dust was taken in 250ml Erlenmeyer flask and was added in the at a concentration of 0.5(v/v).

2.2. Enzyme extraction

From the fermented dough 50mm citrate buffer (pH5) (1:10) was added and homogenized for 2 hours with a constant stirring at room

temprature. This suspention was filtered through whatman filter paper number 1 and the filtarate was again centrifnged at 6000 rpm for 15 min.This solid free supernatant was used as enzyme source activity[7].

2.4. Glucoamylase assay

Glucoamylase activity was determined by following the method.Appropriate amount of the enzyme was reacted with 1% soluble starch solution in 50mm 2-morpholinoethane sulphonic acid(MES) buffer (pH=5.5) at 40ºcfor 40min.the reaction was quenched by placing tubes in boiling water bath for 5min,and then immediately cooled ice.The released glucose was measured using a glucose oxidase method one unit of enzyme activity was defined as the amount of enzyme required to liberated 1mml of glucose equivalent (ml-min) at pH=5.5 and 40ºc. The enzyme activity was given as units per gram of dry substrate (U/g) [8].

2.5. Effect of pH Glucoamylase enzyme production

The effect of pH on enzyme production in different substrates by adjusting the pH of basal salt solution to 4, 5.5, 6.5, 7.7, and 9.The substrate were then incubated for 6 days at room temperature[9].

2.6. Effect of temperature on Glucoamylase enzyme production

The effect of different temperature on enzyme production flasks containing medium kept at temperature range was varied from 30°C, 37°C, 40°C and 50°C with the rise in temperature and the result was recorded[10].

2.7. Effect of carbon and nitrogen

The effect of carbon source on enzyme production was investigated by supplementing the basal salt solution, pH with 2% of different carbon sources such as glucose, maltose, lactose, sucrose and starch the substrate were then incubated for 6 days at room temperature.

The effect of nitrogen sources on enzyme production was the nitrogen sources in basal salt solution,died by replacing pH7,with 2% of $NaNo_3$ (NH_4) SO_4, NH_4CL, NH_4NO_3 and incubated at room temperature for 6 days[11].

2.8. Satistical Analysis

Mean was calculated to facilitate the comparison of data of various physical parameters of enzyme production using various substrate[12].

3. RESULT AND DISCUSSION

The fungal species were isolated from the soil sample and they were identified. This fungal species *Aspergillus niger* and *Aspergillus flavus* was used for the production of glucoamylase by submerged fermentation using rice bran and saw dust.

3.1. Effect of pH on glucoamylase production

In *Aspergillus niger* maximum glucoamylase production was noticed in rice bran at pH_4 (1.16±0.76 IU) followed by saw dust (0.89±0.06 IU) and mixture of rice bran and saw dust (0.84±0.05 IU) (Table 1). In *Aspergillus flavus* maximum glucoamylase production was noticed in rice bran at pH_8 (0.86±0.07 IU) followed by saw dust (0.76±0.73 IU) and mixture of rice bran and saw dust (72±0.06 IU) (Table-2).

In this study maximum enzyme productivity was recorded at pH range 4 same time, highest value was noted in *Aspergillus niger* (1.16±0.76IU) compared with *Aspergillus flavus*.

The similar result was reported as solid state fermentation of starchy materials with *Aspergillus* species were previously reported by many investigators [13] and by endophytic fungi. Endolystic fungus *Colletotrichum gloeosporioides* has greatn capacity of producing amylolytic enzymes (α-amylase and glucoamylase) through fermentations in rice-based solid state fermentation.

3.2. Effect of Temperature on glucoamylase production using

In *Aspergillus niger* maximum glucoamylase production was noticed in rice bran at 25ºc (0.91±0.07 IU) followed by saw dust (0.81±0.06 IU) and mixture of rice bran and saw dust (0.72±0.03 IU) (Table-3) In *Aspergillus flavus* maximum glucoamylase production was noticed in rice bran at 37℃ (0.83±0.09 IU) followed by saw dust (0.73±0.06 IU) and mixture of rice bran and saw dust (0.70±0.06 IU)(Table-4).

In this study maximum enzyme productivity was recorded at temperature range 25℃ and 37℃ same time, highest value was noted in *Aspergillus niger* at 25℃ (0.91±0.07IU) compared than *Aspergillus flavus* at 37℃ (0.83±0.09IU).

It is reported that best enzyme production in *Aspergillus niger* at room temperature both in solid state and submerged fermentation [14] and reported 30°C to be the best for enzyme production by *Pencillium fellutanum*.

3.3. Effect of Substrate concentration on glucoamylase production

In *Aspergillus niger* maximum glucoamylase production was noticed in rice bran at 5gm (0.096±0.07 IU) followed by saw dust (0.71±0.07 IU) and mixture of rice bran and saw

Table-1: Effect of pH on glucoamylase production using *Aspergillus niger*

Organism	Substrate	pH IU/ml			
		4	6	8	10
A.niger	Rice bran	1.16±0.76	0.63±0.05	0.27±0.04	0.19±0.03
	Sawdust	0.89±0.06	0.33±0.08	0.33±0.08	0.24±0.04
	Rice bran + sawdust	0.84±0.05	0.31±0.04	0.31±0.04	0.20±0.05

Results are expressed as mean ± standard deviation

Table-2: Effect of pH on glucoamylase production using *Aspergillus flavus*

Organism	Substrate	pH IU/ml			
		4	6	8	10
A.flavus	Rice bran	0.16±076	0.36±0.05	0.86±0.07	0.53±0.08
	Sawdust	0.89±0.08	0.33±0.08	0.76±0.73	0.46±0.08
	Rice bran + sawdust	0.84±0.04	0.30±0.04	0.72±0.06	0.40±0.08

Results are expressed as mean ± standard deviation

Table-3: Effect of Temperature on glucoamylase production using *Aspergillus niger*

Organism	Substrate	Temperature IU/ml			
		25℃	30℃	32℃	37℃
A.niger	Rice bran	0.91±0.07	0.79±0.06	0.58±0.05	0.46±0.09
	Sawdust	0.81±0.06	0.48±0.05	0.40±0.07	0.33±0.04
	Rice bran + sawdust	0.72±0.03	0.42±0.05	0.38±0.06	0.30±0.04

Results are expressed as mean ± standard deviation

Table-4: Effect of Temperature on glucoamylase production using *Aspergillus flavus*

Organism	Substrate	Temperature IU/ml			
		25℃	30℃	32℃	37℃
A.flavus	Rice bran	0.26±0.05	0.43±0.05	0.46±0.07	0.83±0.06
	Sawdust	0.36±0.05	0.56±0.08	0.63±0.10	0.73±0.06
	Rice bran + sawdust	0.36±0.05	0.51±0.04	0.60±0.08	0.46±0.06

Results are expressed as mean ± standard deviation

Table-5: Effect of Substrate concentration on glucoamylase production using *Aspergillus niger*

Organism	Substrate	Substrate concentration IU/ml			
		5 gm	10 gm	15 gm	20 gm
A niger	Rice bran	0.96±0.07	0.51±0.02	0.63±0.03	0.73±0.08
	Sawdust	0.54±0.05	0.57±0.03	0.62±0.03	0.71±0.07
	Rice bran + sawdust	0.52±0.05	0.50±0.02	0.58±0.03	0.67±0.07

Results are expressed as mean ± standard deviation

Table-6: Effect of Substrate concentration on glucoamylase production using *Aspergillus flavus*

Organism	Substrate	Substrate Concentration IU/ml			
		5 gm	10 gm	15 gm	20 gm
A flavus	Rice bran	0.37±0.05	0.89±0.12	0.50±0.08	0.18±0.08
	Sawdust	0.36±0.07	0.12±0.05	0.17±0.12	0.24±0.07
	Rice bran + sawdust	0.30±0.06	0.11±0.03	0.15±0.05	0.20±0.02

Results are expressed as mean ± standard deviation

dust (0.67±0.07 IU) (Table-5) In *Aspergillus flavus* maximum glucoamylase production was noticed in rice bran at 10gm (0.89±0.12 IU) followed by saw dust at 5gm (0.36±0.07 IU) and mixture of rice bran and saw dust (0.30±0.06 IU)(Table-6).

The maximum enzyme production was recorded at 5 gm, same time highest value was noted in *Aspergillus* niger (0.96±0.07IU) compared than *Aspergillus flavus* using 10gm (0.89±0.12IU).

Similar observation were reported for glucoamylase from Aspergillus niger, *Aspergillus flavus* and *Aspergillus oryzae*[15].

4. CONCLUSION

Glucoamylase producing organism were isolated and identified from the soil sample. *Aspergillus niger*, *Aspergillus flavus* were identified and used for present study. Identified species of *Aspergillus* were screened for glucoamylase producing ability. Then glucoamylase production were analysed in agricultural waste such as a substrate such as rice bran, saw dust and mixture of rice barn and saw dust.Among the study *Aspergillus niger* has high enzyme activity in rice bran compared to *Aspergillus flavus.*In the current study, the optimum conditions for the isolated organisms for glucoamylase production were studied under varying condition of pH such as 4, 6, 8, 10 and temperature (such as 25℃, 30℃, 32℃, 37℃). The maximum production of glucoamylase occur at the temperature 25℃, pH 4 substrate concentration is 5g using *Aspergillus niger*.Finally concluded that the *Aspergillus niger* has highest level of glucoamylase production which was recommended for industrial level glucoamylase production. It was concluded that the nature of the substrate incubation time, temperature, pH etc, all influence the production of glucoamylase in submerged fermentation of rice bran .The results provide valuable information for the production of glucoamylase by *Aspergillus* using relativity inexpensive substrate rice bran. As well as optimization and improvement of process parameters carried out in this study proved to be fruitful in enhancing programs for enzyme of biotechnological important.

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