CULTIVATION OF PENICILLIUM EXPANSUM ON RICE HUSK POWDER FOR PROTEASE PRODUCTION

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SUMMARY : Penicillium expansum link was grown on 1% rice husk fine powder (40 mesh) medium and along with 1% glucose, sucrose, raffinose, maltose, molasses and cornsteep liquor for the production of protease. Production of protease was found to be higher in the rice husk mineral medium and rice husk incorporated with cornsteep liquor as compared to other media. Pure sugars used as carbon and energy sources promoted the growth of Penicillium expansum to varying degrees in comparison to protease production.

Key Words : Penicillium expansum, protease.

INTRODUCTION

Annually, millions of tons of carbon in term of CO_2, are transformed photo-synthetically into plant materials. Approximately one third to one half of the organic matter produced is cellulose. The recycling of this cellulose is brought in nature by microorganisms. Rice husk a major cellulose waste product of rice factories, can through microbial degradation, be converted to fermentable sugars which can be used for production of single cell protein, amino acids, organic acids, antibiotics and enzymes. *Penicillium expansum* is one of the important microorganism taking part in such biodegradation processes by virtue of its ability to produce cellulolytic enzymes (10,14,15).

Penicillium expansum link grow well on a variety of cellulosic materials including textiles and papers but is best known as the cause of distinct brown rot of apples in storage and also to lesser extent in number of soft fruits (17). *Penicillium expansum* is reported to produce pectinase, protopectinase (11), amylase (1,6), celluloses (4,10,14,15), invertase (3), phospholipase (5), lipase (7) on natural and synthetic culture media. In this study the versatile substrate accepting characteristic properties of *Penicillium expansum* was therefore exploited for the production of protease using rice husk as a carbon and energy source under submerged culture condition.

The purpose of this communication is to report the influence of different carbon sources alone and along with combination of rice husk on protease production by *Penicillium expansum.*

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MATERIALS AND METHODS

Strain

Penicillium expansum stain CMI 39761 was used which was obtained from the Department of Botany. University of Glasgow, U.K. The stock culture was maintained at 27°C on agar slants containing 2% bactoagar, 1% peptone and dextrose.

Basal medium

Basal medium was used for the growth of *Penicillium expansum* as reported by Burrel *et. al.* (2), without altering chemical composition, containing following reagents per liter of solution :

Glucose 10.0g; $(NH_4)SO_4$.2.5 g; fumaric acid 2.0 g; KH_2PO_4.1.0 g; $MgSO_4.7H_2O$ 0.5 g; (NH4) Fe(SO4)2.12H2O 0.2

mg; ZnSO4.7H2O 0.2 mg; MnSO4.H2O 0.1 mg and thiamine hydrochloride 0.1mg.

Inoculum

A spore suspension of the fungus was prepared by adding sterile distilled water to stock culture to get $50x10^6$ spores/ml.

Cultivation condition

Fifty ml of basal medium with and without 1% glucose, sucrose, raffinose, maltose, molasses and cornsteep liquor incorporated with 0.5 g of rice husk fine powder (40 mesh) was taken in 250 ml conical flasks. The pH of the medium was adjusted to 6.0 before sterilization. The flasks were plugged with cotton wool and autoclaved at 1.5 kg/cm^2 for 20 minutes. The sterilized media cooled at room temperature were inoculated with 0.5 ml of inoculum containing $50x10^6$ spores/ml. However, the pure sugars were sterilized separately and added aseptically before inoculation. These flasks were incubated at 27±2°C in cooled orbital shaking incubator (Gallenkamp) adjusted at 220 rev per minute. The mycelial biomass was separated from culture broth by filtration through Whatman No.1 filter paper after an interval of 24 hours incubation period.

Determination of protein

Protein content of the culture broth was determined by the method of Lowry *et. al.* (13), with bovine serum albumin as a standard.

Determination of pH values

The initial (before sterilization) and final pH values of culture broth were determined by WPA pH meter (MPA Scientific Instruments).

Determination of mycelial biomass

The quantity of the mycelial biomass was noted after washing with distilled water and drying at 105°C in a hot oven until a constant weight was obtained.

Table 1: Effect of different carbon sources on the growth and protease enzyme production by *Penicillium* expansum.

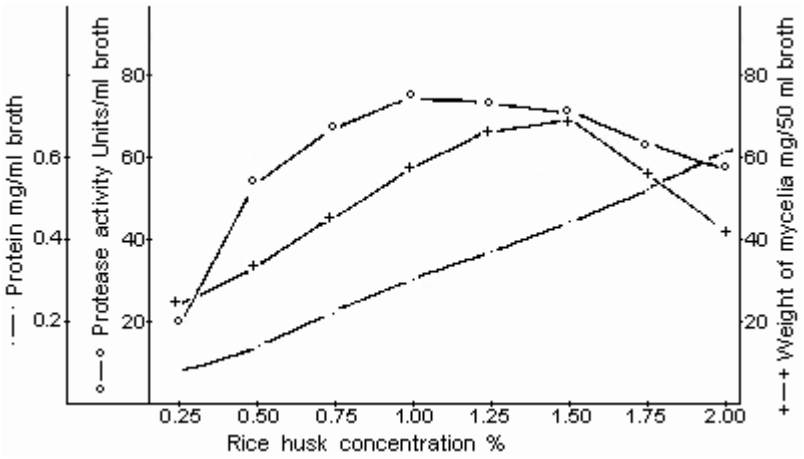
Carbon source	24 hours			48 hours			72 hours			96 hours			120 hours		
	Activity	pH**	*** Mycelia	Activity	pH	Mycelia	Activity	pH	Mycelia	Activity	pH	Mycelia	Activity	pH	Mycelia
Rice husk	40	6.2	30	76	6.5	65	35	6.7	120	26	8.0	160	15	7.85	140
Defatted rice husk	14	6.1	40	36	6.35	80	12	6.55	130	09	6.7	100	04	6.8	60
Oil of rice husk	19	6.15	45	35	6.6	90	17	6.85	140	12	6.7	110	06	6.6	80
Corn steep liquor	13	6.55	60	35	6.9	110	68	7.2	180	42	8.4	210	27	8.2	250
Corn steepliquor+rice husk	20	6.2	50	49	6.45	90	66	6.85	160	90	7.05	200	62	6.8	240
Glucose	06	5.8	70	11	5.1	165	25	4.35	270	30	3.7	360	18	3.2	430
Glucose+Corn steepliquor	05	6.35	65	09	7.3	130	13	7.6	210	08	8.0	280	03	7.8	340
Glucose+rice husk	13	5.9	55	38	5.5	155	15	4.0	190	10	3.95	260	07	3.6	320
Molasses	08	5.6	60	11	4.7	140	16	5.2	235	09	5.7	340	06	5.9	390
Molasses+rice husk	15	5.8	50	50	5.2	90	60	5.6	165	43	5.95	190	20	6.4	240
Sucrose	16	6.35	85	20	6.1	180	33	5.55	290	13	5.25	380	06	5.1	460
Sucrose + rice husk	23	6.45	60	40	6.8	130	14	5.9	200	11	5.35	270	07	5.25	340
Raffinose	04	6.1	55	10	5.9	120	06	5.4	180	04	3.9	250	02	3.4	310
Raffinose + rice husk	09	6.4	40	25	6.2	90	13	5.9	140	08	5.2	170	05	4.1	210
Maltose	08	5.8	75	10	5.5	150	12	5.10	210	09	4.8	290	07	4.4	370
Maltose + rice husk	20	5.9	50	23	5.6	110	17	5.4	160	12	5.05	200	10	4.6	240

* Protease activity Unit/ml culture broth.

** Final pH of the medium, initial pH was adjusted at 6.0.

*** Weight of mycelia mg/50 ml culture broth.

Figure 1: x-axes: Effect of rice husk concentration on the production of protease by Penicillium expansum. y-axes: o - o Protease activity Units/ml broth + - + weight of mycelia mg/50 ml broth, · - · Protein mg/ml broth.



Determination of protease activity

Protease activity in the culture broth was determined by the method of Penner and Aston in combination with that of Lowry *et. al.* (13).

To 1.0 ml of culture broth 3.0 ml of phosphate buffer pH 7.2 and 1.0 ml of casein solution (0.1% in pH 7.2) were added and incubated at 35°C for one hour.

To 2.0 ml of above reaction mixture 2.0 ml of 15% Trichloro acetic acid was added and the precipitates were removed by filtration through Whatman No.1 filter paper.

In 1.0 ml aliquot of above filtrate 4.0 ml of 0.5 N sodium hydroxide and 1.0 ml of Folin phenol reagent (1:1 v/v) was added and total volume was made up to 10.0 ml with distilled water. The blue color developed was determined after five minutes at 625 mμ.

One unit of the protease activity was defined as the amount of enzyme required to release 1 mg of tyrosine under the standard assay conditions described above.

RESULTS AND DISCUSSION

Protease production by *Penicillium expansum* at different concentration of rice husk fine powder (40 mesh) ranging 0.5 to 2% in the basal medium were tried and results are presented in Figure 1. It was observed that maximum protease production and mycelial biomass was obtained at 1.0% and 1.5% rice husk fine powder respectively.

However, further increase of rice husk concentration decreases the protease and mycelial biomass production. It is reported that higher concentration of carbon source in the culture medium suppress the production of enzyme (9,12,18). In subsequent experiments therefore 1.0% rice husk fine powder was used as a carbon source for protease production by *Penicillium expansum.*

The possibility of sugars influencing effect on protease production was investigated and results are compiled in Table 1. It was observed that protease activity 76 units/ml broth or 7600 units/g rice husk fine powder at 48 hours which is greater than pure sugars used as a carbon source either alone or supplemented with rice husk fine powder. However, the yield of protease production was increased to 18.4% at 96 hours when rice husk fine powder supplemented with cornsteep liquor in (1:1) ratio. This may be suggested due to the substrate combination, providing the necessary nutrients which stimulates protease production. Protease production by *Penicillium expansum* was affected considerably by pure sugars. The utilization of pure sugars as carbon and energy source result in good growth with low protease production. These results are greatly supported with the findings of other workers in case of

enzyme synthesis (16,19). They have suggested that carbon source which appeared to poorly utilized energy source for growth, synthesized much larger amount of enzyme than the carbon source used for energy purpose.

The results obtained (Table 1) show that there is no definite relationship between time of growth and extra-cellular protease production. Protease formed at different growth phases may differ in their chemical nature such as protease accumulated in the medium in early growth phase decreased in the later phase of growth may be explained that neutral protease is digested by alkaline protease during later phase of growth (7). This was further substantiated by the finding of Yanagita (20) in case of protease production by *Aspergillus niger*.

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