Screening of Fungi for Single Cell Protein and Cellulase Production

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Isolation and screening for cellulolytic microorganisms for the production of single cell protein and cellulases were carried out. 26 cellulolytic cultures isolated were found to belong to different genera such as **Aspergillus, Fusariumr, Penicillium, Trichoderma and Trametes.** *The isolates were compared for cellulase and protein production with cultures obtained from three different laboratories. The protein content of the products produced by these cultures from alkali-treated rice straw was in the range of* 7.11—41.09 *per-cent, the carboxy methyl cellulase activity (Cx activity) was in the range of* 0-260 *units/ml and filter paper activity (C_1 activity) was in the range of* 0-25 *units/ml. Cultures St-T-M-1 (***Aspergillus carneus***) and 10a (***Trametes sp.***) gave a slightly higher cellulase activity than the cultures* **T. viride** *QM-9123 and* **A. terreus** 6365. *By subjecting the values of protein percentage and enzyme activities to a statistical correlation test, it was observed that there was a positive correlation of* $r = 0.5472$.

The search for new sources of protein is under active investigation and development in several laboratories in the world. Generally, the substrates are starch, hydrocarbons and more recently cellulosic material. The quest is for renewable sources of substrates and in this, cellulose is of prime importance. Protein from these unconventional sources is intended to supplement those from already developed conventional sources. The two possibilities of converting cellulose to single cell protein are (i) directly growing the organism on cellulose for biomass production and (ii) production of cellulase enzymes which can be used for saccharification of cellulosic substrates prior to the cultivation of yeasts or bacteria on them. Use of different strains of fungi for cellulase production is well known (2 & 3) and fungal mycelia are an acceptable source of edible protein (5 & 7). The present paper describes the isolation and screening of some fungal strains for their ability to utilize cellulosic materials for SCP production as well as enzyme production.

Materials and Methods

Fungi were isolated by enrichment technique. Samples of decaying plant materials such as leaves, wood, straw and also soil in the vicinity of decaying matter were collected and added to enrichment flasks having alkali-treated straw (ATS)—mineral medium. After incubating for 7 days a small amount of this broth was transferred to fresh enrichment flasks. After 4 to 5 such serial transfers a dilute suspension of the broth from the last flasks was made and Plated out on Potato Dextrose Agar medium. After incubating the PDA plates for 2 to 4 days at 27°C individual colonies were isolated and subcultured on a modified pDA medium having 0.01% yeast extract and 0.5% dextrose (instead of 2%). These cultures were maintained on the same medium by monthly transfers to fresh medium. The cultures were incubated at 27°C and stored at 4°C.

The standard cultures of *Trichoderma viride* QM-9123 and *T. viride* QM-6a have

been obtained from U. S. Army Natic Laboratories, Natic, Mass. and *Pestalotiopsis versicolor* 6319, *Sporotrichum pruinosum* QM-381, and *Aspergillus terreus* 6365 were obtained from Institute of Fermentation, Osaka, Japan.

Two cultures, *Penicillium chrysogenum* St-F-3B and *Pestalotia* sp. were obtained from National Collection of Industrial Microorganisms, National Chemical Laboratory, Poona.

All these cultures were also maintained in the same way as mentioned above. The cultures isolated in this laboratory were identified by the accepted techniques and procedures (1 & 4).

Medium : For testing the ability of the organisms to produce protein from cellulosic substrates a mineral medium having the following composition was used.

Chemical	gm/L	Chemical	Mg/L
$(NH_4)_2 SO_4$	1.180	$FeSO_4.7H_2O$	4.90
KH_2PO_4	0.904	$MnSO_4.H_2O$	1.86
Urea	0.536	$CoCl_2.6H_2O$	2.01
NaCl	0.508	$ZnSO_4.7H_2O$	1.68
$CaCl_2.2H_2O$	0.110	$CuSO_4.5H_2O$	0.20
$MgSO_4.7H_2O$	0.050		

Alkali-treated rice straw (ATS) was incorporated as the cellulosic substrate at 1 % level. This was prepared by treating the rice straw with 4% NaOH (1 : 50 W/V) and steaming it for 60 minutes. This was washed free of alkali, dried at 55°C and ground.

pH of the medium was adjusted to 5.0 before autoclaving. A mineral medium used by Reese, *et al.* for cellulase production (6) was used for growing the organisms for screening their extracellular cellulase activity. A pure cellulose powder (100-200 mesh) (Biochemical Unit, VPCI, Delhi-110 007) was added at 1% level as carbon source. pH of the medium was adjusted to 5.0 before autoclaving.

Cultivation and harvesting : 100 ml portions of the media along with the substrate were taken in 500 ml. Erlen-Meyer flasks and autoclaved at 15 p.s.i. for 20 min. After cooling the flasks were inoculated by spore suspensions and were incubated on a rotary shaker (230 r.p.m.) at room temperature (23-28°C)

In the case of screening for protein production the contents of the flask were harvested after 72 hrs. incubation by filtering and washing them through previously dried and weighed filter paper (Whattman No.1). This was dried at 55°C and weighed and the product was analysed for total protein conent (N × 6.25) by the micro-Kjeldahl method.

Cellulase activities were estimated in the culture filtrates on 6th and 13th day of incubation.

C_x activity was determined by adding 1 ml of appropriately diluted enzyme to 1 ml of 1 % carboxymethyl cellulose prepared in 0.1 M sodium citrate buffer (pH 4.8) and incubating at 50°C for 30 min. and estimating the liberated reducing sugar by Dinitro-salicylic Acid Method (8).

C_1 activity was estimated by taking 50 mg (1 × 6 cm., coiled) Whatman No. 1 filter paper (FP) in 1 ml. of 0.1 M sodium citrate buffer at pH 4.8 and 1 ml. of appropriately diluted enzyme and incubating for 60 min. at 50°C. The reducing sugar liberated was estimated by DNS method. The number of units per ml. culture filtrate in each case equals the inverse of the dilution required to give 0.5 mg of reducing sugar as glucose.

Results and Discussion

All the organisms tested were found to be capable of growing on ATS mineral medium and the percentage crude protein in the final product ranged from 7.11 to 41.09 (Table 1). 23 organisms out of 33 tested gave a product having more than 15% crude protein.

Aspergillus canerus St-T-M-1 grew well and produced 147 mg crude protein per gm. substrate used, the percentage being 36. All the 4 strains of *A. flavus* gave a product having more than 16% protein, the range being 16.45 to 24.5%. Out of 8 A. *niger* strains 3 produced more than 20% protein, the maximum being 25.8%. *A. terreus* 6365 gave 32.22% protein whereas our isolate gave about 22%. Out of 8 *Fusarium* strains tested only 3 produced about 20% protein. Others produced protein in a range of 10 to 16%. The growth of *P. chrysogenum* St-F-3B was similar to that of the standard cellulolytic culture *T. viride* QM 9123, the protein percentage being 40.5 and 41.09 respectively. The total protein produced per gram substrate was far higher in the former case (180 mg) than the latter (154.8 mg.). One of the other 2 *Penicillium* spp. also has grown well. *Pestaloziopsis versicolor* 6319 gave 26.67 % protein whereas *Pestalotia* sp. gave only 19.3% protein. *Sporotrichum pruinosum* QM-381 did not grow well (protein 7.11%). Standard *T. viride* strains gave high protein percentage (QM-9123 - 41.09 % and QM-6a - 34.02%). Our isolate *Trichoderma* sp. 10b also gave 29% protein. A basidiomycete, *Trametes* sp. 10a gave 18% protein.

Of the 33 cultures studied (including 5 standard cellulase producing cultures) the following isolates showed extracellular enzyme activity. They were *A. carneus* ST-T-M-1, *A. flavus* IRS-5, *A. niger* strains IP-2, RLM-11, C-1, ALS-1 and RLM-6, *A. terreus* ISt-1, *Fusarium* sp. IRS-3 and IRS-7, *Trichoderma* sp. 10b and *Trametes* sp. 10a. Standard organisms *T. viride* QM-9123 and *T. viride* QM-6a showed an activity of 200 and 128 units of C_X and 19.2 and 28 units of filter paper (FP) activities respectively, whereas our isolates *Trametes* sp. 10a, *A. carneus* ST-T-M-1, *A. terreus* ISt-1, *A. niger* C-1, and *Trichoderma* sp. 10b showed Cx activities of 260, 230, 100, 86 and 40 units per ml. and FP activities of 25, 12, 8, 4 and 4 units/ml respectively. There has been no reports on *Aspergillus carneus* as a source of cellulase enzymes.

In some of the cases, we observe, that even though the organism was capable of utilizing the cellulose powder and showed growth, concurrent formation of extracellular enzyme could not to detected.

The values of enzyme activities (Cx) and the protein percentage values (biomass in ATS medium) were subjected to a correlation test. It was observed that there was a slight positive correlation of $r = 0.5472$.

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Table 1 – Production of mycelial protein by different fungi from alkali-treated rice straw

Sl. No.	Name of the organism		Final pH of the medium	Weight of biomass + residual substrate mg/gm substrate	Protein (%)	Total protein mg/gm substrate
1.	*Aspergillus carneus*	St-T-M-1	3.2	473	36.02	146.79
2.	*Aspergillus flavus*	IRS-5	6.2	467	24.50	114.42
3.	do	ICC-2	3.9	634	20.80	131.87
4.	do	ALS-3	3.7	606	19.77	119.80
5.	do	ALS-7	3.5	680	16.45	111.86
6.	*Aspergillus niger*	IP-2	2.4	466	25.80	120.23
7.	do	RLM-5	2.3	512	25.58	130.97
8.	do	RLM-11	2.5	597	21.18	130.20
9.	do	C-1	2.3	584	19.84	115.87
10.	do	ALS-1	2.5	572	13.72	78.48
11.	do	IOB-1	3.2	654	11.69	70.77
12.	do	RLM-6	2.5	667	11.57	77.17
13.	do	ISt-2	3.0	696	7.44	51.78
14.	*Aspergillus terreus*	6365	4.3	433	32.22	139.41
15.	do	ISt-1	5.6	568	21.95	124.68
16.	*Fusarium* sp.	IRS-3	4.0	57[illegible]	20.89	119.49
17.	do	ALS-6	3.7	695	19.27	133.92
18.	do	RLM-4	4.8	518	18.93	98.16
19.	do	IRS-7	4.0	548	16.28	89.21
20.	do	ALS-5b	3.8	665	13.85	92.10
21.	do	IRS-9	5.0	452	13.72	61.91
22.	*Fusarium* sp.	ALS-5a	3.9	660	10.45	68.97
23.	do	IRS-15	4.8	595	10.12	60.21
24.	***Penicillium chrysogenum***	**St-F-3B**	**3.5**	**445**	**40.50**	**180.00**
25.	*Penicillium* sp.	IP-1	4.4	550	23.86	131.87
26.	do	ALS-2	4.0	660	13.72	90.55
27.	*Pestaloziopsis versicolor*	6319	4.0	348	26.67	92.81
28.	*Pestalotia* sp.		6.5	401	19.31	77.43
29.	*Sporotrichum pruinosum*	QM-381	3.8	767	7.11	54.53
30.	*Trichoderma viride*	QM-9123	2.7	377	41.09	154.80
31.	do	QM-6a	2.6	412	34.02	140.16
32.	*Trichoderma* sp.	10b	6.0	378	28.24	106.75
33.	*Trametes* sp.	10a	2.4	615	18.00	110.07

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