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SCREENING OF MICROORGANISM FOR INDUSTRIAL PRODUCTION OF CELLULASE

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ABSTRACT
The study was to screen various microorganisms obtained from various laboratories situated in and around Chennai, Totally 73 species were grown in the culture medium. After the growth the mycelial dry weight, Protein content and cellulase activity were established. From this *Alternaria*, *Bacillus*, *Aspergillus*, *Trichoderma*, *Fusarium* and *Penicillium* shows good result for cellulose production. Among them, *Trichoderma viride* and *Aspergillus terreus* were recorded as cellulase producing species. Production of cellulase was analyzed by Dinitrosalicylic acid (DNS) and Carboxymethyl cellulase assay methods. Maximum enzyme production (1.76 U/ml) was achieved at 30°C, pH of 5.0 by *Aspergillus terreus* and on 6th day of incubation. When the culture medium was supplemented with Banana leaves, Rice husk, Millet husk, coir waste, wheat bran, saw dust, Rice straw and ground nut banana leaves (725 units) shows optimum production. Among various percentages 6% shows highest (825units) cellulose production.

KEY WORDS: *Cellulase, Carboxy Methyl Cellulose, Screening, Substrate concentration, Enzyme Production.*

INTRODUCTION

Cellulase is a nature polymer found exhaustively amount in plant cell walls. This is also produced by some animals like tunicates and few bacteria. It is an industrial important hydrolytic enzymes and of great significance in present day Biochemistry and Biotechnology. It is widely used in the food, feed, textile, and pulp industries (Nakari and Pentilla, 1996). A Cellulase enzyme also has novel application in manufactured such as butanol, methane, ethanol, Single cell proteins, amino acids, paper, rayon, cellophane, production and processing of chemicals and extensively utilized for extraction of valuable components from plant cells preparation of plant protoplast in genetic research and improvement of nutritional value of animal feed (Kadar et al., 1999), Mandels, 1985). The enzyme cellulase have also used for feed preparation , food processing, detergent formulation, textile production, production of wine, beer and fruit juice, waste water treatment and in other areas (Jahangeer *et al*., 2005, Walsh, 2002, Philippidis, 1994). Several microorganisms are capable of degrading cellulose only few microorganisms produce significant amount of cell free enzymes capable of hydrolyzing crystalline cellulose *in vitro*. Among to this fungi are the main cellulose producing microorganism. Recently some bacteria and actinomycetes have also been found producing cellulases. Considering the importance of these cellulases to the industry, the present study is aimed to screen various microorganisms obtained from Chennai, Bangalore, and Vellore research institutes and screened for large scale production of cellulases in our Laboratory

MATERIALS & METHODS

Cleaning of glass wares, maintenance of the microorganisms, preparation of potato dextrose agar (PDA) medium, Sub culturing, preparation of glasswarefor sterilization, natural medium and pectin medium, Mycelial dry weight determination and methodologies were adopted according to the methods of Annadurai *et al*. (1989, 96, 98, 99, 2000). Glass distilled water was used for the preparation of the media, reagent solutions and for final rinsing of all glass wares. Cellulase production by various microorganisms, Culture media, Culture methods, Inoculation of the medium, Mycelial dry weight, Fungal Biomass were done according to the Methods of Maragathavalli and Annadurai (2015). The cellulase enzyme activity was done according to the method of Ghosh 1987 and Bateman (1966). Statistical analyses were done according to Baily (1984) and Radhakrishna Rao *et al*. (1985).

RESULTS

Isolation of fungal strains from different sources, their identification and screening for Cellulase enzyme production

To pick potent strains of microrganisms capable of degrading Cellulose, 73 strains obtained from different laboratories culture collections were screened. Out of these 12 belongs *Fusarium*, 4 *Alternaria*, 20 *Aspergillus*, 13 *Penicillium*, 3 *Mucor*, 4 *Bacillus*, 3 *Rhizopus*, 13 belongs to other fungi. These fungi belong to 20 genera but most of the active strains belonged to the genus *Aspergillus* group. Literature reports also support this findings (Raper and Fennel, 1965; Reed, 1966) It is also found that almost all fungal strains, irrespective of their source and their taxonomic position were capable of attacking Cellulose even though the degree of attack varied. Ragheb and Fabian (1955) also have reported that all the species of fungi tested by them possessed Cellulose degrading ability. There were also differences in the kind

of Cellulose degrading enzymes produced by different strains of the 73 strains screened 25 proved to be highly Cellulolytic, and out of these 20 belonged to the black *Aspergillus* group. As mentioned earlier, it is reported that strains of *Aspergillus* are generally utilized for commercial production of the enzyme. Thus US Food and Drug Administration (FAD) also permits enzymes produced by *Aspergillus* group only to be used in food processing. The FAD laws state that Cellulase prepared in accordance with "good manufacturing practice" as defined by the FAD and derived from *A. niger* are "generally recognized as safe" (GRAS). Finally, a strain of *Aspergillus* was selected which was found to give maximum enzyme yield both in liquid and solid media. Particular strain of microorganism is very important from industrial point of view. Bateman and Millar, 1966 was of the opinion that it is strain rather than the species which is important for selecting an organism for producing Cellulase enzymes. Davies (1963) also reports that it is the strain rather than the species belonging to genera, that it is important in selecting an industrial microorganism. He is also if the view that all enzymes even in uninduced state.

Other workers have also screened a number of isolates for selecting a potent culture (Domozych *et al*., 2004) screened 11 *Rhizopus* sp. For Cellulase production and observed marked variation between the Cellulase but *R. nigrieans* secreted very little. Kertesz, 1955, also reported that fungal strains varied in the rate and nature of decomposition of Cellulose when they tested soil microorganisms. Dingle and Solomans (1952) found that out of 113 microfungi tested, three belonging to the *Aspergillus* group. Mycelial dry weight, Estimation of Protein and Cellulase enzymes were done as described in Materials and Methods.

TABLE 1: Mycelial dry weight, protein content and cellulase activity of different microorganisms in culture filtrate

S.NO	Microflora	df	pH	Protein content		Mycelial dry		Cellulase activity	
				mg±sd	Si	mg±sd	Si	mg ± sd	Si
1	*Acremonium kilense*	5	2.76	0.22± 0.024	+	0.128±0.004	N	0.35±0.01	+
2	*Acrocylinarium sp*	5	2.76	0.14± 0.036	N	0.168±0.0025	N	0.326±0.012	+
3	*Alternaria temissinea*	5	2.87	0.38± 0.007	+	0.445±0.0054	++	0.582±0.00008	++
4	*Alternaria tenius*	5	2.87	0.47±0.002	+	0.559±0.015	++	0.568±0.00002	++
5	*Alternaria triticina*	5	2.87	0.42±0.004	+	0.753±0.044	++	0.591±0.00002	++
6	*Alternaria triticola*	5	2.87	0.39±0.006	+	0.902±0.077	++	0.513±0.00089	++
7	*Aspergillus aureus*	5	2.47	0.6±0.001	+	0.126±0.004	N	0.716±0.0036	++
8	*Aspergillus nidulans*	5	2.87	0.5±0.003	+	0.225±0.0045	++	0.715±0.004	++
9	*Aspergillus niger*	5	2.87	0.4±0.002	+	0.1180.0052	++	0.512±0.0008	++
10	*Aspergillus oryzae*	5	2.87	0.4±0.004	+	0.245±0.0038	++	0.690±0.005	++
11	*Aspergillus terreus*	5	2.87	0.48±0.006	+	0.275±0.0042	++	0.815±0.004	++
12	*Aspergillus awamori*	5	2.47	0.55±0.008	+	0.461±0.0065	+	0.784±0.008	++
13	*Aspergillus candidas*	5	2.47	0.58±0.002	+	0.162±0.02	N	0.771±0.007	++
14	*Aspergillus citreus*	5	2.47	0.58±0.002	+	0.121±0.005	N	0.787±0.0085	++
15	*Aspergillus flavus*	5	2.47	0.64±0.0009	+	0.165±0.002	N	0.817±0.0112	++
16	*Aspergillus foetidusi*	5	2.47	0.56±0.0002	+	0.11±0.005	N	0.658±0.0012	++
17	*Aspergillus fumigatus*	5	2.47	0.59±0.0008	+	0.176±0.002	+	0.804±0.01	++
18	*Aspergillus tamari*	5	2.47	0.62±0.0005	+	0.143±0.003	N	0.866±0.016	++
19	*Aspergillus ochraceus*	5	2.47	0.62±0.0005	+	0.166±0.002	N	0.779±0.008	++
20	*Aspergillus oryzae*	5	2.47	0.56±0.0002	+	0.185±0.0018	++	0.845±0.014	++
21	*Aspergillus parasiticus*	5	2.47	0.48±0.0016	+	0.113±0.005	N	0.792±0.008	++
22	*Aspergillus saitoi*	5	2.47	0.59±0.008	+	0.213±0.09	N	0.78±0.008	++
23	*Aspergillus sojae*	5	2.47	0.63±0.0072	+	0.178±0.002	N	0.751±0.0061	++
24	*Aspergillus tarrarii*	5	2.47	0.32±0.012	+	0.514±0.01	++	0.772±0.007	++
25	*Aspergillus wenti*	5	2.47	0.59±0.0008	+	0.293±0.0002	N	0.764±0.006	++
26	*Aspergillus versicolar*	5	2.47	0.47±0.002	+	0.219±0.0007	N	0.755±0.0061	++
27	*Bacillus licheniformis*	5	7.48	0.76±0.007	+	0.146±0.003	N	0.624±0.00038	++
28	*Bacillus subtilis*	5	7.48	0.78±0.008	+	0.161±0.002	N	0.641±0.00074	++
29	*Bacillus*	5	7.48	0.72±0.004	+	0.162±0.002	N	0.655±0.001	++
30	*Bacillus thuringiensis*	5	7.48	0.68±0.002	+	0.156±0.003	N	0.692±0.002	++
31	*Endothia parasitica*	5	6.06	0.32±0.012	+	0.168±0.0025	N	0.486±0.0017	+
32	*Fomitopsis sp*	5	2.87	0.05±0.006	+	0.143±0.0072	++	0.245±0.006	++
33	*Fusarium mali*	5	6.84	0.46±0.002	+	0.274±0.0007	N	0.454±0.0031	+
34	*Fusarium solani*	5	2.87	0.35±0.005	+	0.178±0.0038	++	0.423±0.006	++
35	*Fusarium candidum*	5	6.84	0.48±0.001	+	0.145±0.003	N	0.445±0.0036	+
36	*Fusarium didymum*	5	6.84	0.52±0.0005	+	0.142±0.003	N	0.467±0.0025	+
37	*Fusarium discolor*	5	6.84	0.55±0.0008	+	0.155±0.003	N	0.458±0.0029	+
38	*Fusarium gibbosum*	5	6.84	0.62±0.0005	+	0.177±0.002	N	0.449±0.0034	+
39	*Fusarium giberella*	5	6.84	0.46±0.002	+	0.305±0.0012	+	0.489±0.0016	+
40	*Fusarium moniliformis*	5	6.84	0.44±0.003	+	0.185±0.001	N	0.452±0.0031	+
41	*Fusarium nivale*	5	6.84	0.42±0.004	+	0.399±0.002	+	0.433±0.0043	+
42	*Fusarium orthoceras*	5	6.84	0.46±0.002	+	0.128±0.004	N	0.449±0.0034	+

43	*Fusarium oxysporum*	5	6.84	0.63±0.007	+	0.548±0.014	++	0.461±0.0028	+
44	*Fusarium subulatum*	5	6.84	0.44±0.003	+	0.33±0.005	+	0.47±0.0026	+
45	*Mailmalbronchia*	5	2.86	0.22±0,24	+	0.235±0.0004	N	0.492±0.0015	+
46	*Mucor hiemalis*	5	3.25	0.38±0.007	+	0.464±0.006	+	0.534±0.00042	++
47	*Mucor parasitica*	5	3.25	0.37±0.008	+	0.867±0.068	++	0.589±0.00016	++
48	*Mucor pusillus*	5	3.25	0.35±0.0089	+	0.144±0.003	N	0.545±0.00024	++
49	*Paecilomyces varioti*	5	2.28	0.28±0.016	+	0.136±0.004	N	0.325±0.012	+
50	*Penicillium brasilianum*	5	2.87	0.03±0.004	+	0.224±0.0039	++	0.345±0.005	++
51	*Penicillium occitanis*	5	2.87	0.05±0.003	+	0.234±0.0042	++	0.387±0.004	++
52	*Penicillum chrysogenum*	5	2.75	0.59±0.0008	+	0.264±0.0005	N	0.545±0.0036	++
53	*Penicillum citrinum*	5	2.75	0.55±0.0008	+	0.624±0.023	++	0.434±0.0043	+
54	*Penicillum cumemberti*	5	2.75	0.62± 0.005	+	0.267±0.0005	N	0.489±0.0016	+
55	*Penicillum dubontil*	5	2.75	0.48± 0.001	+	0.277±0.0007	N	0.548±0.0002	++
56	*Penicillum expansum*	5	2.75	0.65± 0.001	+	0.256±0.0011	N	0.526±0.00058	++
57	*Penicillum fungoculom*	5	2.75	0.58± 0.0002	+	0.368±0.001	+	0.542±0.0028	++
58	*Penicillum griseofulvin*	5	2.75	0.56± 0.002	+	0.235±0.004	N	0.428±0.0046	+
59	*Penicillum janthinellum*	5	2.75	0.45±0.002	+	0.474±0.007	++	0.589±0.00016	++
60	*Penicillum notatum*	5	2.75	0.52±0.0005	+	0.241±0.003	N	0.561±0.0072	++
61	*Penicillum roqueforti*	5	2.75	0.46±0.002	+	0.255±0.0001	N	0.57±0.00002	++
62	*Penicillum stoloniferum*	5	2.75	0.47±0.002	+	0.242±0.003	N	0.549±0.0019	++
63	*Pleurotus ostreatus*	5	2.87	0.05±0.003	+	0.162±0.0041	++	0.324±0.003	++
64	*Rhizopus chinensis*	5	6.76	0.34±0.01	+	0.285±0.0005	N	0.718±0.003	++
65	*Rhizopus nigericans*	5	6.76	0.36± 0.008	+	0.299±0.0007	N	0.682±0.002	++
66	*Rhizopus oligosporus*	5	6.76	0.32±0.012	+	0.233±0.0003	N	0.691±0.002	++
67	*Saccharomyces*	5	3.55	3.76±2.03	+	0.318±0.0002	+	0.318±0.013	+
68	*Scrytalidium linicolum*	5	2.18	0.38±0.007	+	0.153±0.003	N	0.386±0.007	+
69	*Strptococcus*	5	2.87	0.14±0.036	N	0.245±0.0003	N	0.344±0.0108	+
70	*Strptomyces rectus*	5	2.84	0.28± 0.016	N	0.359±0.001	+	0.405±0.005	+
71	*Trametessan guinea*	5	2.76	0.28± 0.016	N	0.269±0.0005	N	0.291±0.016	NS
72	*Trichoderma reesei*	5	2.87	0.06±0.004	+	0.326±0.023	++	0.810±0.003	++
73	*Tritirachium album*	5	2.67	3.17± 1.35	+	0.368±0.001	+	0.418±0.005	+

Fig 1.a.Protein content, Mycelial dry weight and Cellulase Activity of *Alternaria Sp*

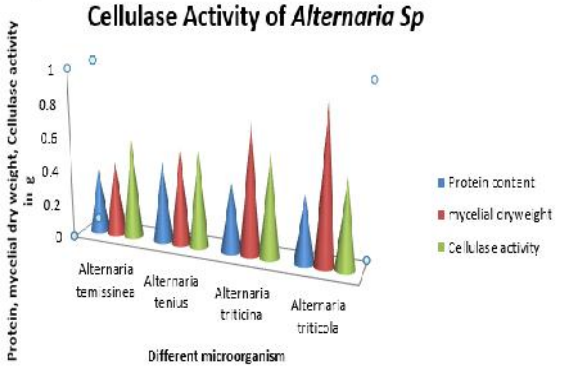


Fig.1.b.Mycelial dry weight,Protein Content,Cellulase Activity of fusarium

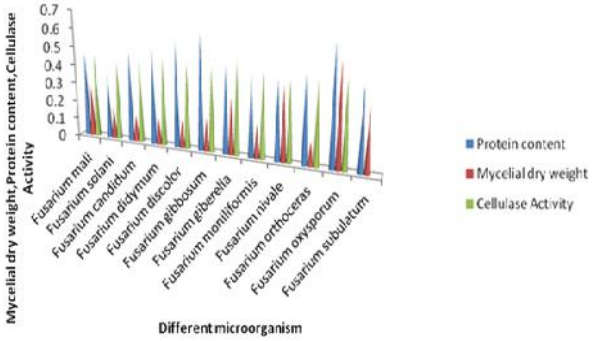


Fig.1.c.Mycyelial dry weight, Protein Content, Cellulase Activity of *Aspergillus spp*

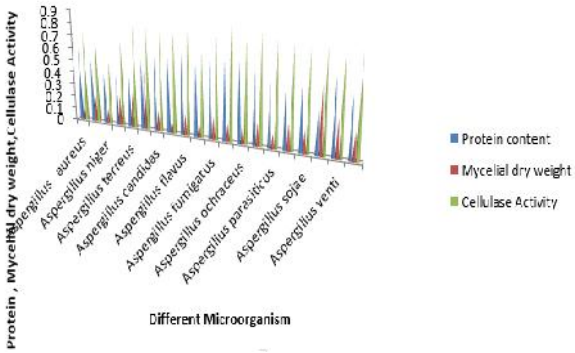
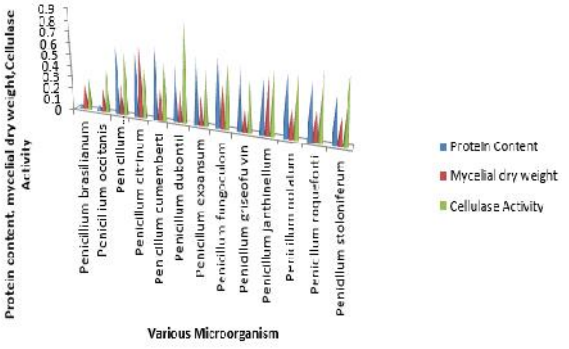
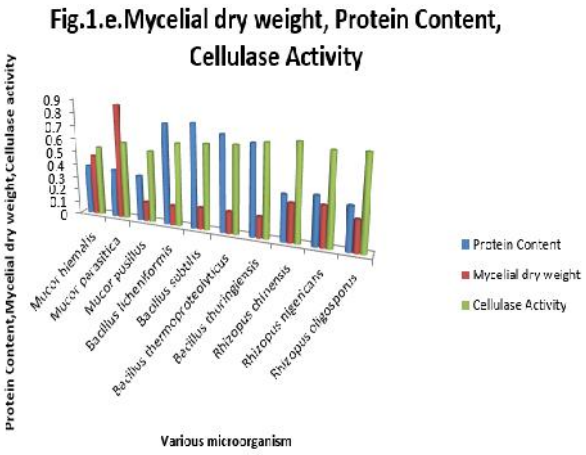
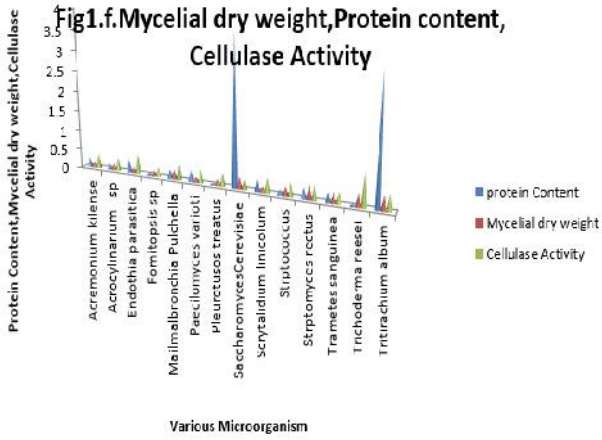


Fig.1.d.Mycelial dry weight, protein Content, Cellulase Activity in *Penicillium spp*







RVU=1000/t_1, Where t is the time taken for 50% reduced of viscosity.

One unit of endoglucanase activity was defined as the amount of enzyme releasing one µ mole of reducing sugar /ml /h

Values given are the mean value ($\bar{X}$) of 4 datas

d.f. = degrees of freedom = n-1

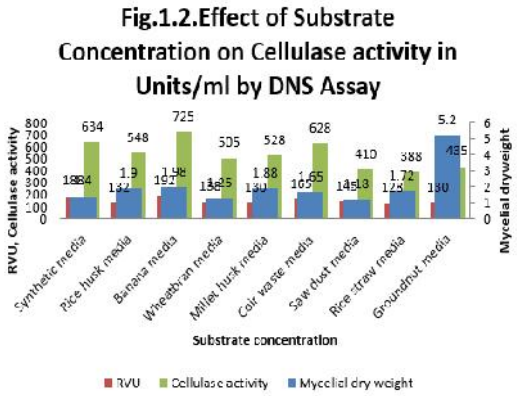
Significance ++ = $p < 0.001$

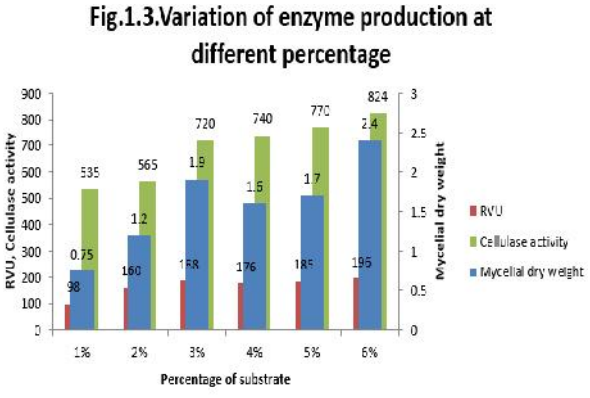
\+ = $p < 0.05$

NS = Not significant

Table. 1 shows mycelial dry weight protein content and cellulase activity in different microorganisms. Fig 1a shows the mycelial dry weight, Cellulase activity and protein content in *Alternaria* species. In this mycelial dry weight was maximum in *Alternaria triticola* whereas protein and cellulose content was equal in all the species. Figure 1b indicates dry weight, Cellulase activity and protein content in *Fusarium* species. *Fusarium oxysporum* and *Fusarium mali* shows maximum cellulose activity. Fig1c presents mycelial dryweight, Cellulase activity and protein content in *Aspergillus.* Out of all this *Aspergillus terreus* shows maximum cellulose activity.Fig1d explains mycelial dry weight, Cellulase activity and protein content in *Penicillium*. In this *Penicillium dubantill* shows the highest activity of cellulase. Fig 1e critically explains mycelial dry weight, Cellulase activity and protein content of *Bacillus, Mucor and Rhizopus*. *Mucor* shows maximum mycelial weight and *Rhizopus* shows maximum cellulose activity. The mycelial dry weight was maximum in *Bacillus subtilis, Bacillus licheniformis, Fusarium, Rhizopus etc*. the protein content was maximum in *Bacillus, Penicillum.* The cellulase activity was maximum in *Aspergillus* and *Trichoderma* and *Alternaria* species. Fig 2, presents the effect of Cellulase production in different culture media. In this Nine media were tested the relative viscosity units and DNS method of cellulase assay are presented .Out of all these media Banana peel media and Millet husk media shows maximum production, whereas other media are less significant ($p<001$). In many microorganisms sequential production of Cellulase enzyme were reported. (Vries, R.P., Visser, J., 2001; Vega K, *et al*., 2012, Mohammed Inuwa Ja'afaru, 2013),

Enzyme multiplicity and diversity between the *invivo* and *invitro* patterns have been observed in many cases. Our results concur with the results of Bateman, 1972, Cervone *et al*., 1977, Arinze and Smith 1979.Variation of enzyme production at different period is presented in table .3 the relative viscosity unit and reducing sugar method significantly shows at the 3rd week of growth of cellulase activity. Afterwards it shows less activity. These results concur with result of Alkorta *et al*., 1998.





Substrate concentrations of 1 % to 6 % were considered for the production of cellulose (Fig 3). It was observed that the banana peels, rice husks and wheat bran gave the higher production at 3% substrate concentration. After that second highest production was obtained from Millet husks, coir waste and saw dust at 4% substrate concentration. And the lowest production was obtained from the maize cobs at 4% substrate concentration. The

decrease in activity beyond maximum substrate concentration that is 5% may be due to the inhibitors. This is supported by the findings of Gbekeloluwa and Moo-young (1991), who reported the inhibitory effect of accumulated cellobiose and cellodextrin of low degree of polymerization.

DISCUSSION

A total number of 73 microbial samples collected from different laboratories of Tamilnadu. Cellulases are the enzymes which hydrolysis cellulosic biomass and are being produced by the microorganisms grown over cellulosic matters. Cellulase is an important enzyme which can be obtained from microorganisms, as well as cellulose as substrates by using submerged fermentation and solid state fermentation. Cellulose protein can be degraded by cellulase enzyme produced by cellulolytic bacteria and fungi (Lekh Ram *et al*., 2014, Sudarshan Singh Rathore, 2014). This enzyme has various unique industrial applications and it has been considered as major group of industrial enzyme from various region including different research laboratories. Total 73 isolates were obtained by the primary screening technique from which 11 isolates were showing maximum cellulase activity. These 11 isolates were then evaluated by secondary screening for enzyme production. Among these 1 isolate was selected as most efficient enzyme producers and their specific enzyme activity in the crude sample was found to be 6.0U/mg and 8.4 U/mg and of partially purified sample was found to be 6.97 U/mg and 9.3 U/mg respectively. Isolates were tentatively characterized on the basis of their cultural and morphological and biochemical characteristics. They were identified to be *Aspergillus terreus* (Lekh Ram *et al*., 2014, Sudarshan Singh Rathore, 2014). Further partial purification of the cellulase enzyme was carried out by ammonium sulfate precipitation followed by dialysis. Optimization of different parameters was carried out for the production of cellulase by both efficient isolates. The maximum enzyme producing isolate *Aspergillus* was used to check biodegradation properties at laboratory scale.

Cellulase characteristics and production by *Aspergillus* spp. have been well documented in the literature (Lockington *et al*., 2002; Ong *et al*., 2004; Wang *et al*., 2008). However, only a few reports are available on the production of cellulase by *Aspergillus terreus* (Emtiazi *et al*., 2001; Gao *et al*., 2008; Pushalkar and Rao, 1998; Singh *et al*., 1996), and in many cases, have not been studied in depth. The microorganisms which appear to be most promising at present are *Aspergillus* sp. and *Trichoderma* sp. However, it is of interest to examine *Aspergillus* sp. to improve cellulase production which is a known good producer of cellulases (Jecu *et al*., 2000, Sharada, R. *et al*., 2012). Many researchers have been conducted on enzymatic hydrolysis of various lignocellulolytic substrates like Pumpkin oil cake, Saw dust, Pine apple waste, Orange waste, Palm oil mill effluent, pea shrub biomass, Sugarcane bagasse, Rice bran, Rice straw, wheat bran, vinegar waste, Cassava waste, Corn straw, wheat straw, rice husk, soybean, cotton, corn cob, green grass, dried grass, Millet, Oats straw, Oil palm biomass, Banana stalk, mulch, Radicle waste (Padmavathi *et al*., 2012). On the basis of the above study it was concluded that, the selected fungal strains have the ability to degrade the cellulosic wastes, out of the all fungal strains *Aspergillus terreus* is the more efficient for the degradation of cellulose. The uses of fungal strains for the enzyme productions have many advantages such as, the enzymes produced are normally extracellular, making easier for the extraction process. The used Dinitro salicylic acid (DNS) method for the assessment of reducing sugars was suitable for routine analysis of reducing sugars. The present study was aimed at the condition optimization for the production of cellulase by using various agricultural wastes. And the Banana peels, Rice husks and Millet husks gave the higher production of the cellulase. In the present study, it could be concluded that the fungal cultures obtained from different places possess cellulolytic activity. Among these fungal cultures, *Aspergillus terraeus* was noticed to show maximum zone of hydrolysis (4.2 cm) of carboxy methyl cellulase assay (CMCase (64 U/ml), biomass (1560 mg/ml) and extracellular protein content (1.65 mg/ml). The fungal cultures isolated in the present investigation need to be further studied in depth for their cellulolytic potential for conversion of cellulosic waste material into useful products.

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