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RESEARCH ARTICLE

Isolation, Purification and Screening of Cellulolytic Fungi from Mushroom Compost for Production of Enzyme (Cellulase)

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ABSTRACT

In the present study compost samples of two mushrooms namely Buttton (*Agaricus bisporus)* and Shiitake (*Lentinula edodes*) were collected from Directorate of Mushroom Research (DMR) ,Chambaghat, Solan, Himachal Pradesh, India. After screening cellulase producing fungi a total of 45 fungal isolates were isolated from compost samples, out of which 36 fungal isolates were purified and 23 isolates showed the cellulase activity. The isolates were identified as *Trichoderma, Aspergillus, Rhizopus* and *Penicillium* species. The optimum pH and temperature for growth and enzyme production were found to be 6.0 and 28°C respectively. The maximum fungal growth and enzyme production by *Aspergillus* spp. was obtained after 4 days of incubation period whereas *Trichoderma*, *Penicillium* and *Rhizopus* species exhibited maximum fungal growth and enzyme production after 5 days of incubation. Sucrose was found to be the best carbon source in *Trichoderma* spp. and *Penicillium* species at 1 percent concentration, whereas Glucose exhibited maximum enzyme activity in case of *Rhizopus* spp. and *Aspergillus* spp. at 1.5 percent and 1 percent concentration resspectively. One percent peptone proved to the best nitrogen source in case of *Trichoderma* spp. Yeast extract exhibited maximum enzyme activity in case of *Rhizopus* spp. at 1 percent concentration, whereas Beef extract at 1 and 1.5 percent concentration was found to be best nitrogen source in case of *Aspergillus* and *Penicillium* species respectively.

INTRODUCTION

Cellulases were initially investigated several decades back for the bioconversion of biomass which helps in the industrial application of enzymes in animal feed, food textiles, and detergents and in the paper industry (Cen and Xia, 1999). With the shortage of fossils fuels and the arising need to find alternative source for renewable energy. Cellulose is considered as one of the most important sources of carbon on this planet and its annual biosynthesis by both land plants and marine algae occurs at a rate of 0.85×10^{11} tonnes per annum (Nowak *et al*., 2005). The value of cellulose as a renewable source of energy has made cellulose hydrolysis the subject of intense research and industrial interest (Bhat, 2000; Coral *et al*., 2002) It is considered to be an exhaustible source of raw material for different products (Klemn *et al*., 2002). Cellulolytic fungi which are capable of growth on insoluble cellulose, only a small number of these produce extracellular enzymes that can degrade cellulose (Mandles and Weber, 1969). Microbial degradation of lignocellulosic waste and the downstream products resulting from it is accomplished by a concerted action of several enzymes, the most prominent of which are cellulases, which are produced by a number of microorganisms. Cellulolytic microbes are primarily carbohydrate degraders and are generally unable to use proteins or lipids as an energy source for growth (Lynd *et al*., 2002). Microbial utilization of the inexhaustible cellulosic biomass for the production of industrial chemicals and preparation of cellulose polymers will help to meet energy and food demands. Such potential applications could help solve modern waste disposal problems, help to alleviate shortage of food, animal feed and diminish man's dependence on fossil fuels (Cowling and Kirk, 1976; Ghosh and Singh, 1993). The growing concerns about the shortest of fossils fuels, the emission of green house gases and air pollution by incomplete combustion of fossils fuels has also resulted in an increased focus on production of bioethanol from lignocelluloses and especially the possibility to use cellulases and hemicellulases to perform enzymatic hydrolysis of the lignocelluloses material (Himmel *et al*., 1999; Zaldivar, 2001).

Cellulases are used in biostoning of denim garments for producing softness and faded look of denim garments replacing use of pumice stones which were traditionally employed in industry (Bhat, 2000; Olson and Stanley, 1991). *Humicola insolans* cellulase is most commomnly employed in biostoning, through use of acidic cellulase from *Trichoderma* along proteases (Cortez *et al*., 2001). Cellulases are also used in detergents for cleaning textiles. Several reports (Fowler, 2000; Nielsen, 1994; Clarkson, 2000) disclose that the *Trichoderma ressei* are suitable for the use in detergents. Cellulase preparations mainly from the species of *Humicola* that are active under mild alkaline condition and elevated temperature are commomnly added in washing powder (Mitchinson and Wendt, 2001) and in detergents (Uhlig, 1998). In the food industry, cellulases are used in extraction and clarification of fruit and vegetable juices, production of nectars and purees, and extraction of olive oil (Galante and Monteverdi, 1998). In paper and pulp industry, cellulases have been employed for pulping for modification of coarse and hand sheet strength properties (Bedford *et al*., 2003; Akhtar, 1994) delinking of recycled fibres (Pere *et al*., 1995) and improving drainage and runnability of paper mills (Prasad *et al*., 1992).

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MATERIALS AND METHODS

The samples of mushroom compost were collected from Directorate of Mushroom Research (DMR), Chambaghat, Solan, Himachal Pradesh. Isolation of mesophilic fungi was done with enrichment technique. Serial dilutions of compost samples were made. One ml solution was taken from the enriched broth and serial dilution with distilled water was carried out. Solutions with 10^{-2}, 10^{-4}, 10^{-6} dilutions were plated on petri plates containing cellulose agar media. The plates were incubated at 28° C for 5 days to isolate fungal colonies. The colonies showing significant fungal growth were selected and stabbed on fresh petri plates containing Potato Dextrose Agar medium. The pure culture of fungi were made by hyphal tip method and maintained at 4°C for further use. The fungal isolates from Button mushroom compost were designated as BM and those isolated from Shiitake mushroom compost were designated as SM, respectively. The cultures were identified based on the microscopic and macroscopic characteristics. Lactophenol cotton blue staining was performed for microscopic observation. A drop of distilled water was placed on a clean slide. Small tuft of the fungus preferably with spores and spore bearing structures was transferred into the water drop using a flamed, cooled needle. Drop of lacto phenol cotton blue stain was added over the material and was mixed. The slides were observed under low and high power objectives of the microscope. The fungal isolates were assigned code numbers, and enriched in the nutrient broth and were further screened for assay of cellulase activity. The mushroom compost associated fungi were tested for their ability to produce the cellulase enzyme by using cellulose agar plates to detect cellulase activity of fungi. The isolated fungi were stabbed on the solidified agar and allowed to incubate for 48 hrs (to express cellulose depolymerization through cellulase production into its surrounding medium). The plate were stained with 0.1% Congo red / Gram's Iodine solution and counterstained with 1M NaCl for 15-20 min for decolorization zone observation.

Production of Cellulase

The seed culture (50ml) was prepared by inoculating a bit of pure culture from slant into seed medium. The seed medium used was given by Mandel and Reese (1957). 2ml of seed culture was inoculated in 50 ml of production medium. Composition of production medium was same as seed medium. The flasks were incubated for 5 days in incubation shaker at temperature of 28°C and at 130 rpm. Production medium was centrifuged at 10,000 rpm for 10 min. The supernatant was collected and stored in refrigerator for further use. The purpose of the assay is to check the purity of enzymes but does not measure directly the amount of enzymes. Qualitative assays are powerful tools used in screening fungi for lignocelluloses degrading enzyme production. Such tests give a positive or negative indication of enzyme production. One unit of activity (U) is defined as the amount of enzyme liberating l u mol of reducing sugar per minute in a standard assay. The assay of cellulase enzyme was done according to method given by Ress and Mendel (1963); Milles (1959).

Optimization of culture conditions for enzyme production

Effect of pH on cellulase production

The effect of pH was evaluated by inoculating the flasks containing 50 ml of sterile production medium, with 100 µl seed culture. The inoculated flasks were maintained at selected pH of 5, 6, 7, 8 and 9. The pH of medium was adjusted by using 1N HCl or 1N NaOH. The inoculated flasks were incubated in rotatory shaker incubator at 28°C for 5 days at 130 rpm and centrifuged at 10,000 rpm for 10 min. Pellet was discarded and supernatant was taken for assay of cellulase.

Effect of temperature on cellulase production

The effect of temperature was evaluated by inoculating the flasks containing 50 ml of sterile production medium, with 100 µl seed culture.The inoculated flasks were maintained at different temperature varying from 24°C, 26°C, 28°C, 30°C, 32°C for 5 days at 130 rpm in rotatory shaker and centrifuged at 10,000 rpm for 10 min. Pellet was discarded and supernatant was taken for assay of cellulose.

Effect of Incubation Period on cellulase production

To find out the effect of incubation period on the production of cellulase, the flasks containing 50 ml sterile production medium were inoculated with 100µl seed culture and incubated for 7 days at optimum temperature. The growth and enzyme production rate was measured after 24 hrs intervals.

Effect of carbon sources on cellulase production

The effect of carbon sources on enzyme production was studied at different concentration 0.5%, 1.0%, 2.0% and 2.5% (w/v). Fifty ml of production medium was distributed into flasks and supplemented with different concentration of carbon compounds (Glucose, Sucrose and Lactose). The flasks were then inoculated with 100µl seed culture and incubated in incubator shaker at 28°C for 5 days at 130 rpm and centrifuged at 10,000 rpm for 10 min. The optimum carbon source was found by analyzing the result of cellulase production.

Effect of nitrogen sources on cellulase production

The effect of nitrogen sources on enzyme production was studied at different concentration 0.5%, 1.0%, 2.0% and 2.5% (w/v). Fifty ml of production medium was distributed into flasks and supplemented with different concentration of nitrogen compounds (peptone, beef extract and yeast extract). The flasks were then inoculated with 100µl seed culture and incubated in incubator shaker at 28°C for 5 days at 130 rpm and centrifuged at 10,000 rpm for 10 min. The optimum nitrogen source was found by analyzing the result of cellulase production.

RESULTS AND DISCUSSION

Isolation of mesophilic fungi

Compost samples of two mushrooms viz. Button (*Agaricus bisporus*) and Shiitake (*Lentinula edodes*) were collected from Solan (H.P.). The compost samples of both the mushrooms contained high number of mesophilic fungi. The mesophilic fungal colonies isolated from the mushroom compost samples are detailed in Table 1. It is evidient from Table 2 that out of 45 fungal isolates, 36 fungal isolates were purified for further investigations.

Table 1. Total number of fungal colonies in mushroom compost samples

S.No.	Name of the Sample	Dilution Factor	No. of Colonies
1.	Button mushroom compost (*Agaricus bisporus*)	10^{-3}	31
		10^{-5}	20
2.	Shiitake mushroom compost (*Lentinula edodes*)	10^{-3}	20
		10^{-5}	16

Table 2. Total number of fungal isolates purified from each mushroom compost sample

S.No.	Name of the sample	No. of isolates	Purified isolates
1.	Button mushroom compost (*Agaricus bisporus*)	25	19
2.	Shiitake mushroom compost (*Lentinula edodes*)	20	17

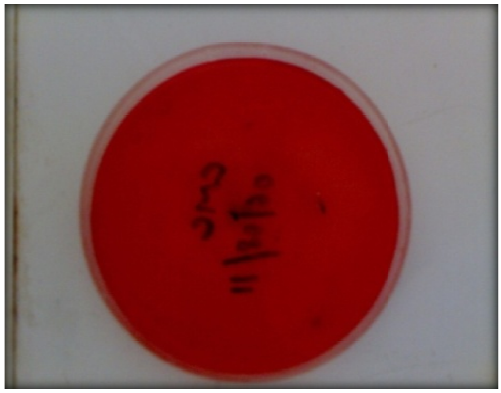
Screening of mesophilic fungi for cellulase activity

Screenings of fungi for cellulase activity were carried out by the hydrolysis of substrate (Carboxy Methyl Cellulose) incorporated in the basal medium. The absence of zone or dark background in Fig. 1 (A) shows negative results, whereas presence of clear zone as

in Fig. 1 (B, C, D) around the fungal growth indicates the positive results. The isolates showing maximum zone(s) were selected for further studies related to cultural and physiological parameters for optimization of enzyme activity. Data (Table 3) depict that only 23 fungal isolates hydrolyzed the cellulose agar.

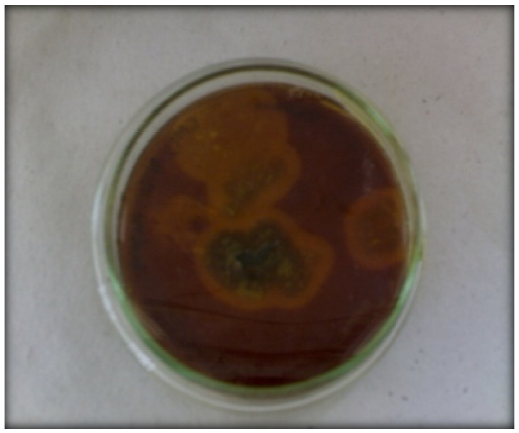
Table 3. Screening of fungal isolates for cellulase production

S.No.	Name Of the sample	Total isolates	Cellulase +ve	Cellulase -ve
1.	Button mushroom compost (*Agaricus bisporus*)	19	12	7
2.	Shiitake mushroom compost (*Lentinula edodes*)	17	11	6



A

B



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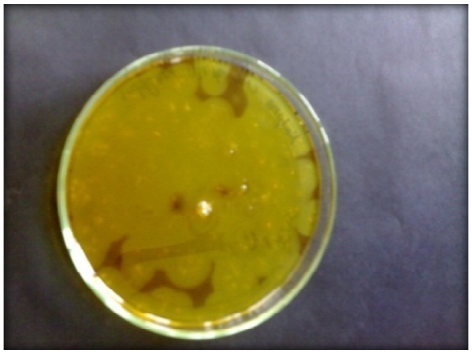

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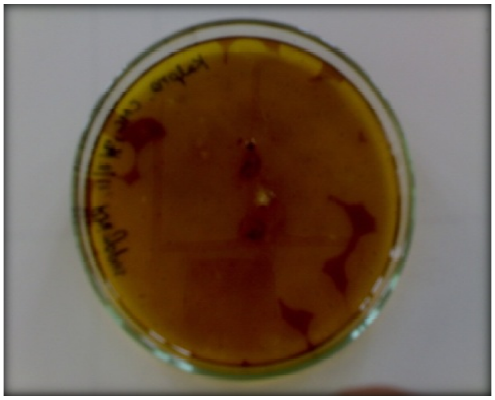

E

Fig. 1 (A). Absence of zone indicating negative result. B, C, D and E Showed zone of hydrolysis by *Trichoderma spp.*, *Aspergillus* spp., *Rhizopus* spp., *Penicillium* spp. Respectively

Morphological Characterization of Fungal Isolates

The identification of cellulase producing fungi was performed by studying their microscopic and macroscopic characters. Lactophenol cotton blue staining was performed so that better microscopic observation(s) could be made. Morphological characters of cellulase positive isolate are shown in Table 4 and 5. Isolates BM2, BM5, BM6, BM8, BM12, SM1, SM2 and SM11 were identified as *Aspergillus* spp. The specie produce colonies, composed of white or yellow felt that is covered by dark asexually produced fungal spores. Conidiophores (asexually produced fungal spores) of *Aspergillus* spp. contain globose (globular) vesicles. Each globose vesicle is completely covered with phialides which are radiating from entire surface. The conidia are one celled, coloured in mass and arranged in basipetal chains. Isolates BM3, BM10, SM4, SM7 and SM9 were identified as *Rhizopus* spp. They grow as white mold in culture. Mycelial or threadlike hyphae are divided by a transparent septum. They produce distinctive conidiophores which are short and simple. The conidia are one celled, ovoid in shape and smooth. Isolate BM1, BM4, BM9, SM3 and SM8 belongs to *Penicillium* spp. The *Penicillium* spp. produces colonies which were cottony white in colour with green tinge. The conidiophores arise from mycelium and are branched. The conidia are smooth and ovoid. Isolates BM7, BM11, SM5, SM6 and SM10 were recognized as *Trichoderma* spp. as they show green colour in culture. Conidiophores of *Trichoderma* spp. are hyaline and branched. It contain phillades which occur singly or in groups. The conidia are coloured in mass and occur in basipetal chains. It is clear from the Table (4 and 5) that the compost samples of both the mushrooms contained high number of mesophilic fungi.

Table 4. Morphological characters of cellulase positive fungal isolates of Button mushroom compost

S.No.	Isolate code	Colour of mycelium	Conidiophore	Conidia
1	BM1	Cottony white with green	Arising from mycelium, branched from apex, penicillate, ending in phillades	Hyaline, brightly coloured in mass, 1-celled globose
2	BM2	Blackish greenish tinge	Upright simple, terminating in globose, bearing phialides at apex	1-celled globose, coloured in mass, basipetal chains
3	BM3	White	Short, simple	1-Celled, hyaline, ovoid and smooth
4	BM4	Cottony white with green	Arising from mycelium, branched from apex, penicillate, ending in phillades	Hyaline, brightly coloured in mass, 1-celled globose or ovoid
5	BM5	greenish tinge	Upright simple, terminating in globose, having phialides radiating from surface	1-celled globose, coloured in mass, basipetal chains
6	BM6	Light greenish tinge	Upright simple, terminating in globose.	1-celled globose, coloured in mass
7	BM7	Green	Hyaline, much branched, phillades single or in groups	Ovoid, 1-celled, borne in clusters, green patches
8	BM8	Blackish greenish tinge	Upright simple, terminating in globose, bearing phialides at apex	1-celled globose, coloured in mass, basipetal chains
9.	BM9	Cottony white with green	Arising from mycelium, branched from apex, penicillate, ending in phillades	Hyaline, brightly coloured in mass, 1-celled globose or ovoid
10.	BM10	White	Short, simple	1-Celled, hyaline, ovoid and smooth
11.	BM11	Green	Hyaline, much branched, phillades single or in groups	Ovoid, 1-celled, borne in clusters, green patches
12.	BM12	greenish tinge	Upright simple, terminating in globose, bearing phialides at apex or radiating from entire surface	1-celled globose, coloured in mass, basipetal chains

Table 5. Morphological characters of cellulase positive fungal isolates of Shiitake mushroom compost

S.No.	Isolate code	Colour of mycelium	Conidiophore	Conidia
1.	SM1	greenish tinge	Upright simple, terminating in globose, bearing phialides at apex	1-celled globose, coloured in mass, basipetal chains
2.	SM2	Blackish greenish tinge	Upright simple, terminating in globose, bearing phialides at apex	1-celled globose, coloured in mass, basipetal chains
3.	SM3	Cottony white with green	Arising from mycelium, branched from apex, penicillate, ending in phillades	Hyaline, brightly coloured in mass, 1-celled globose
4.	SM4	White	Short, simple	1-Celled, hyaline, ovoid and smooth
5.	SM5	green	Hyaline, much branched, phillades single or in groups	Ovoid, 1-celled, borne in clusters, green patches
6.	SM6	green	Hyaline, much branched, phillades single or in groups	Ovoid, 1-celled, borne in clusters,green patches
7.	SM7	White	Short, simple	1-Celled, hyaline, ovoid and smooth
8.	SM8	Whitish greenish	Simple rarely short branches, dark, phillades in chains	Single, apical, globose, brown 1- celled
9.	SM9	White	Short, simple	1-Celled, hyaline, ovoid and smooth
10.	SM10	Green	Hyaline, much branched, phillades single or in groups	Ovoid, 1-celled, borne in clusters, green patches
11.	SM11	Blackish greenish tinge	Upright simple, terminating in globose, bearing phialides at apex or radiating from entire surface	1-celled globose, coloured in mass, basipetal chains

Goyal and Soni (2011) have also reported the screening of fungi for cellulase production from *Pleurotus florida* left over substrate. There are many reports on isolation of cellulase producing fungi from soil (Lalitha, 2011), lignocellulosic waste from the vinegar industry (Liu and Yang, 2007), waste paper, cotton waste, bagasse and leaf litters (Thomas and Ambikapathy, 2011). It is clear from the data (Table 6 and 7) that out of 36 fungal isolates, 23 showed cellulose hydrolysis and maximum cellulase production (2.50 U/ml) was recorded with the fungal isolate SM6 which was identified as *Trichoderma* spp., followed by *Aspergillus* spp. (BM2) (1.99 U/ml), *Penicillium* spp. (BM4) (1.60 U/ml) and *Rhizopus* spp. (SM7) (1.50 U/ml). These four fungi were selected for further investigation as they exhibited highest cellulase activity in decreasing order.

Table 6. Screening of mesophilic fungi isolated from Button mushroom compost

S.No.	Name of fungi with Isolate code	Cellulase (U/ml)
1.	*Penicillium* spp. (BM1)	0.20
2.	*Aspergillus* spp. (BM2)	1.99
3.	*Rhizopus* spp. (BM3)	1.00
4.	*Penicillium* spp. (BM4)	1.60
5.	*Aspergillus* spp. (BM5)	1.20
6.	*Aspergillus* spp. (BM6)	0.40
7.	*Trichoderma* spp. (BM7)	0.10
8.	*Aspergillus* spp. (BM8)	0.10
9.	*Penicillium* spp. (BM9)	1.00
10.	*Rhizopus* spp. (BM10)	0.80
11.	*Trichoderma* spp. (BM11)	0.60
12.	*Aspergillus* spp. (BM12)	0.40

Table 7. Screening of mesophilic fungi isolated from Shiitake mushroom compost

S.No.	Name of fungi with Isolate code	Cellulase (U/ml)
1.	*Aspergillus* spp. (SM1)	0.20
2.	*Aspergillus* spp. (SM2)	1.00
3.	*Penicillium* spp. (SM3)	1.00
4.	*Rhizopus* spp. (SM4)	0.11
5.	*Trichoderma* spp.(SM5)	1.02
6.	*Trichoderma* spp.(SM6)	2.50
7.	*Rhizopus* spp. (SM7)	1.50
8.	*Penicillium* spp. (SM8)	0.30
9.	*Rhizopus* spp. (SM9)	0.50
10.	*Trichoderma* spp. (SM10)	1.30
11.	*Aspergillus* spp. (SM11)	0.01

Optimization of culture conditions for enzyme production

Effect of pH on cellulase production by mesophilic fungi

The pH is one of the most important factors affecting the growth of fungus. In general an enzyme production by fungi is strongly influenced by the pH of the growth medium and vary from species to species (Garg and Neelakantan, 1981; Niranjane *et al*., 2007). Result illustrated in Table 8 shows that irrespective of fungi cellulase production, expressed as enzyme activity, gradually increases with increase in pH and reached its maximum at pH 6, but above and below this pH growth and production of cellulase declined. *Trichoderma* spp. showed maximum enzyme activity (3.42 U/ml) at

pH 6 followed by pH 7 (3.06 U/ml) and minimum enzyme activity was recorded at pH 9 (1.69 U/ml). Effect of pH on cellulase production by the *Trichoderma* spp. supports the findings of Lee *et al*., 2002 who reported pH 6 to be optimum for cellulase production from *Trichoderma viride*. The growth and cellulase production by *Aspergillus* spp. was maximum at pH 6.0 (4.79 U/ml). Above and below this pH there was decline in the enzyme production. The present findings are in confirmatory with the earlier observations made by Abika *et al*., 1995 who reported pH between 6.0 and 7.0 to be optimum for cellulase activity by *Aspergillus niger*. Species of *Rhizopus* and *Penicillium* exhibited maximum enzymatic activity at pH 6.0 (5.83 U/ml and 1.99 U/ml) respectively. Minimum enzyme activity (0.13U/ml) by *Rhizopus* spp. was at pH 5 whereas *Penicillium* spp. exhibited minimum activity (0.25 U/ml) at pH 9. These observations are in agreement with those reported by Milala *et al*., 2009; Das and Ghosh, 2009; Hayano, 1986; Kshattriya, 1992.

Table 8. Effect of pH on the cellulase production by mesophilic fungi

S.No.	pH	Enzyme Activity (U/ml)			
		Trichoderma spp.	*Aspergillus* spp.	*Rhizopus* spp.	*Penicillium* spp.
1.	5	2.80	4.08	0.13	0.75
2.	6	3.42	4.79	5.83	1.99
3.	7	3.06	0.95	2.50	0.89
4.	8	2.80	0.53	2.40	0.76
5.	9	1.69	0.98	2.32	0.25

Effect of temperature on cellulase production by mesophilic fungi

Temperature plays an important role in expressing the activity of any biological system; it has great influence on the production of end product. It is evidient from the data (Table 9) that irrespective of fungal isolates, maximum fungal growth and enzyme production was at 28° C and minimum activity was recorded at 32° C. Maximum cellulase production by *Trichoderma* spp. was found at 28° C (4.70 U/ml), followed by decrease in the enzyme activity with increase or decrease in the temperature from 28° C (Fig. 3). Murao *et al*., 1988, Lu *et al*., 2003; Zhou *et al*., 2008; Yun *et al*., 2001 have reported different temperatures for maximum cellulase production suggesting that the optimum temperature for cellulase production also depends on the type of microorganisms. Similar trend in decline in enzymatic activity on either side of 28° C was observed in *Aspergillus* spp., *Trichoderma* spp., *Penicillium* spp. and *Rhizopus* spp. The studies corroborate to the findings of Milala *et al*., 2009; Singh *et al*., 2009 and Narsimha *et al*., 2006. Amongst the four selected fungi maximum enzymatic activity was shown by *Trichoderma* followed by *Rhizopus* (3.89 U/ml), *Aspergillus* (3.61 U/ml) and *Penicillium* (2.80 U/ml) species.

Table 9. Effect of Temperature on cellulase production by mesophilic fungi

S.No.	Temperature (°C)	Enzyme Activity (U/ml)			
		Trichoderma spp.	*Aspergillus* spp.	*Rhizopus* spp.	*Penicillium* spp.
1.	24	2.89	3.00	2.08	1.50
2.	26	3.50	3.23	3.60	2.01
3.	28	4.70	3.61	3.89	2.80
4.	30	4.00	2.22	2.80	2.10
5.	32	2.80	1.09	2.06	1.11

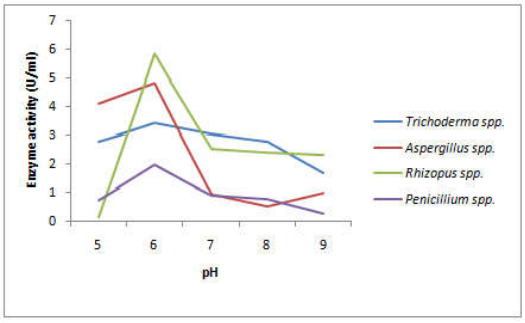

Fig. 2. Effect of pH on cellulase production by mesophilic fungi

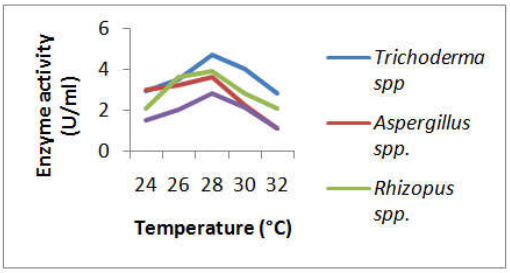

Fig. 3. Effect of Temperature on cellulase production by mesophilic fungi

Effect of incubation period on cellulase production by mesophilic fungi

The incubation period is directly related to the production of enzyme. The maximum fungal growth and enzyme production (1.84 U/ml) by *Aspergillus* spp. was obtained after 4 days of incubation period (fig 4) whereas *Trichoderma* (1.91 U/ml) and *Penicillium* (2.75 U/ml) species exhibited maximum fungal growth and enzyme production after 5 days of incubation. These results are in agreement with Kang *et al*., 2004. Whereas the peak of cellulase activity (1.81 U/ml) by the *Rhizopus* spp. was also obtained after 5 days incubation. This rate of production is similar to those already reported for fungal strains of *Rhizopus* by Acharya *et al*., 2008; Youssef *et al*., 2009; Milala *et al*., 2009.

Table 10. Effect of incubation period on cellulase production by mesophilic fungi

S.No.	Incubation Period (Days)	Enzyme Activity (U/ml)			
		Aspergillus spp.	*Trichoderma spp.*	*Rhizopus* spp.	*Penicillium* spp.
1	1st	1.31	1.45	1.51	1.80
2	2nd	1.49	1.61	1.64	1.86
3	3rd	1.61	1.74	1.71	1.90
4	4th	1.84	1.81	1.75	2.00
5	5th	1.64	1.91	1.81	2.75
6	6th	1.51	1.79	1.71	1.39
7	7th	1.47	1.75	1.65	1.40

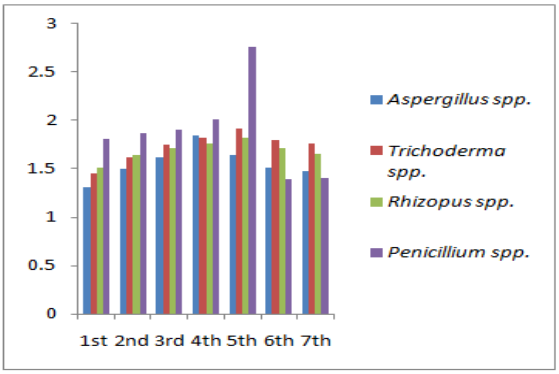

Fig. 4. Effect of incubation period on cellulase production by mesophilic fungi

Effect of carbon sources on cellulase production by mesophilic fungi

The production of cellulase is a key factor in the hydrolysis of cellulosic material and is essential to make the process economically viable. Cellulase production by *Trichoderma* spp. increased with increase in initial sugar concentration from 0.5% to 1.0% (Table 11). Irrespective of carbon sources, highest cellulase production was obtained at 1% concentration, with increase in sugar concentration, reduction in enzyme activity was observed. Mandel and Reese (1957) reported maximum yield of cellulase at 1% concentration with different carbon sources in *Trichoderma viride*. Sucrose was best effective carbon source for cellulase production (13.32 U/ml), followed by glucose (5.16 U/ml) and lactose (4.10 U/ml). Same carbon sources have been optimized by different workers for cellulase production (Sherief *et al*., 2010; Solomon *et al*., 1997; Lee

et al., 2010). A perusal of data (Table 12) showed that enzyme activity of *Aspergillus* spp. was maximum at 1% and minimum at 0.5% concentration irrespective of carbon sources. Lynd *et al*., 2002 reported that with increase in concentration of carbon sources there was decline in the enzymatic activity. It is evident from the data (Table 13) that *Rhizopus* spp. exhibited maximum enzyme activity at 1.5% concentration of different carbon sources. Glucose proved to the best carbon source for the cellulase production (1.91U/ml) and followed by sucrose (1.81 U/ml) and Lactose (1.70 U/ml). The results obtained for *Rhizopus* spp. were contrary to those obtained by Gokhalie *et al*., 1991 who reported that glucose probably acted as the end product inhibitors for cellulase production may be due to type of chemical(s) used, storage and analytical conditions available for undertaking the present investigations. Enzyme activity of *Penicillium* spp. was maximum at 1% concentration irrespective of carbon source (Table 14). In present studies sucrose resulted in maximum enzyme activity (2.50 U/ml), and minimum enzyme activity (1.86 U/ml) was those obtained with glucose at1% concentration.These findings are in agreement with those obtained by Gao *et al*., 2004 who stated that cellulase activity was maximum when sucrose was used as the sole carbon source in case of *Penicillium chrysogenum*.

Table 11. Effect of carbon sources on cellulase production (U/ml) by *Trichoderma* spp.

S.No.	Carbon Sources	Enzyme activity (U/ml) Concentration (%)				
		0.5	1.0	1.5	2.0	2.5
1.	Glucose	1.25	5.16	3.06	1.59	0.89
2.	Sucrose	8.59	13.32	9.23	7.63	4.55
3.	Lactose	2.06	4.10	3.99	2.89	1.06

Table 12. Effect of carbon sources on cellulase production (U/ml) by *Aspergillus* spp.

S.No.	Carbon Sources	Enzyme activity (U/ml) Concentration (%)				
		0.5	1.0	1.5	2.0	2.5
1.	Glucose	1.28	2.75	2.19	1.78	1.41
2.	Sucrose	0.30	1.45	1.10	0.93	0.40
3.	Lactose	0.34	1.92	1.18	0.51	

Table 13. Effect of carbon sources on cellulase production (U/ml) by *Rhizopus* spp.

S.No.	Carbon Sources	Enzyme activity (U/ml) Concentration (%)				
		0.5	1.0	1.5	2.0	2.5
1.	Glucose	0.75	1.65	1.91	0.64	0.53
2.	Sucrose	1.10	1.50	1.81	0.93	0.65
3.	Lactose	0.95	0.65	1.70	0.49	0.40

Table 14. Effect of carbon sources on cellulase production (U/ml) by *Penicillium* spp.

S.No.	Carbon Sources	Enzyme activity (U/ml) Concentration (%)				
		0.5	1.0	1.5	2.0	2.5
1.	Glucose	0.19	1.86	0.89	0.45	0.29
2.	Sucrose	0.68	2.50	1.85	0.75	0.39
3.	Lactose	0.55	1.99	0.99	0.85	0.50

Table 15. Effect of nitrogen sources on cellulase production (U/ml) by *Trichoderma* spp.

S.No.	Nitrogen Sources	Enzyme activity (U/ml) Concentration (%)				
		0.5	1.0	1.5	2.0	2.5
1.	Peptone	3.60	6.50	4.32	3.51	1.30
2.	Yeast extract	1.05	1.83	1.21	0.89	0.51
3.	Beef extract	2.89	4.99	4.01	3.47	2.65

Table 16. Effect of nitrogen sources on cellulase production (U/ml) by *Aspergillus* spp.

S.No.	Nitrogen Sources	Enzyme activity (U/ml) Concentration (%)				
		0.5	1.0	1.5	2.0	2.5
1.	Peptone	1.19	3.72	2.99	1.25	0.89
2.	Yeast extract	0.19	1.62	1.10	0.99	0.91
3.	Beef extract	2.01	4.44	3.25	1.50	0.85

Table 17. Effect of nitrogen sources on production of cellulase (U/ml) by *Rhizopus* spp.

S.No.	Nitrogen Sources	Enzyme activity (U/ml) Concentration (%)				
		0.5	1.0	1.5	2.0	2.5
1.	Peptone	1.25	3.77	2.50	1.50	0.75
2.	Yeast extract	1.99	5.00	2.99	1.79	0.89
3.	Beef extract	0.29	0.74	0.59	0.45	0.29

Table 18. Effect of nitrogen sources on cellulase production (U/ml) by *Penicillium* spp.

S.No.	Nitrogen Sources	Enzyme activity (U/ml) Concentration (%)				
		0.5	1.0	1.5	2.0	2.5
1.	Peptone	2.01	2.95	3.99	1.63	0.96
2.	Yeast extract	3.89	2.89	4.05	2.01	1.95
3.	Beef extract	3.65	2.61	5.03	1.95	1.50

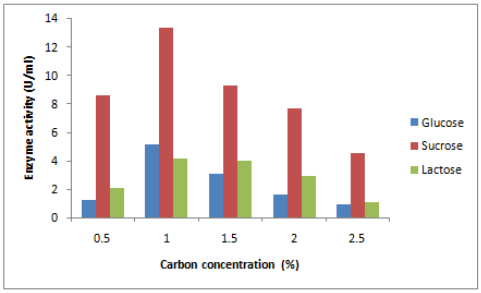

Fig. 5. Effect of carbon sources on production of cellulase (U/ml) by *Trichoderma* spp.

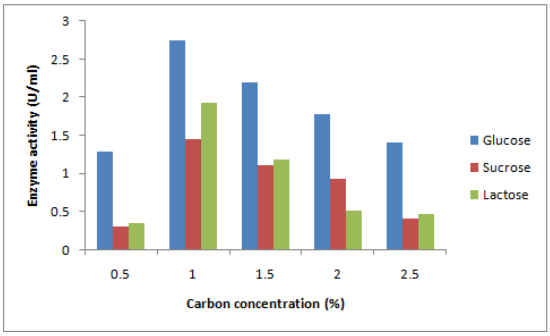

Fig. 6. Effect of carbon sources on cellulase production (U/ml) by *Aspergillus* spp.

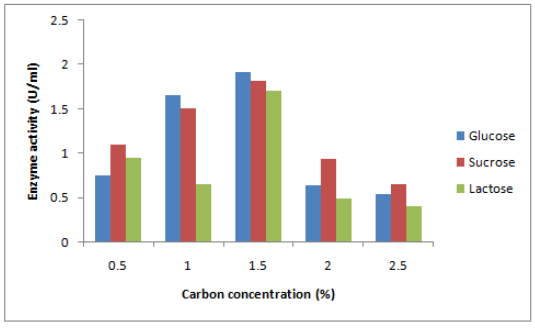

Fig. 7. Effect of carbon sources on cellulase production (U/ml) by *Rhizopus* spp.

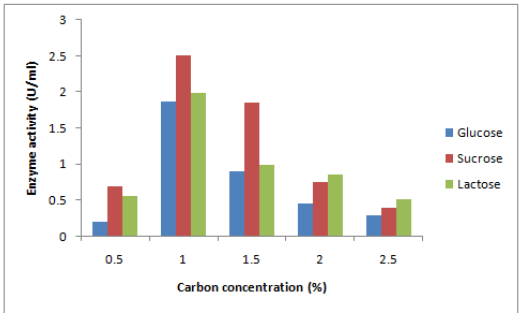

Fig. 8. Effect of carbon sources on cellulase production (U/ml) by *Penicillium* spp.

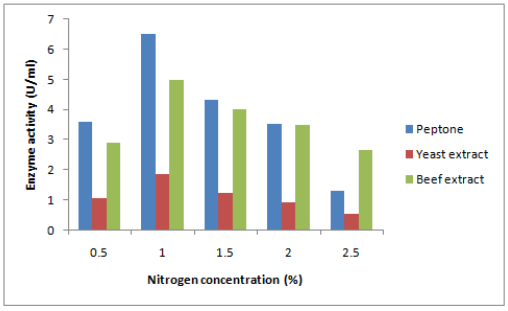

Fig. 9. Effect of nitrogen sources cellulase production (U/ml) by *Trichoderma* spp.

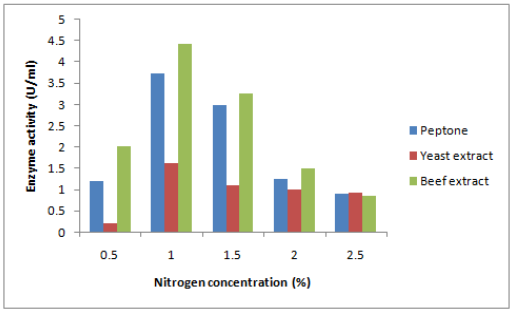

Fig. 10. Effect of nitrogen sources on cellulase production (U/ml) by *Aspergillus* spp.

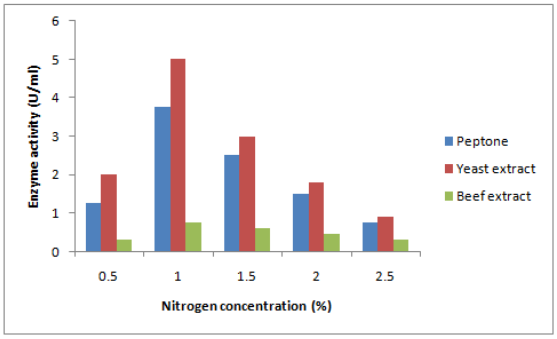

Fig. 11. Effect of nitrogen sources on cellulase production (U/ml) by *Rhizopus* spp.

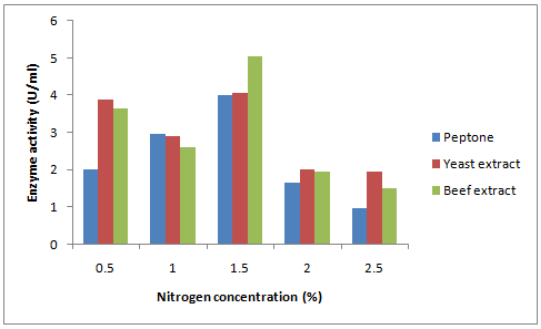

Fig. 12. Effect of nitrogen sources on cellulase production (U/ml) by *Penicillium* spp.

Effect of nitrogen sources on cellulase production

According to Spiridonov and Wilson (1998), all the microorganisms which have an important industrial application can utilize inorganic and organic nitrogen sources. The results depicted in Table (15) showed that in *Trichoderma* spp. the maximum enzyme activity (6.50 U/ml) was obtained with peptone and minimum (1.83 U/ml) with yeast extract. Sun *et al*., 1999 reported that the addition of organic nitrogen sources resulted in increased growth and cellulase production. Data (Table 16) revealed that the supplementation of nitrogen sources at 1% (peptone, Yeast extract, Beef extract) in *Aspergillus* spp. stimulated the cellulase yield and activity. Maximum cellulase activity was obtained when beef extract was used as a nitrogen source (4.44 U/ml) but the minimum enzyme activity was with yeast extract (0.62 U/ml). These findings are more or less similar to Narasimha *et al*., 2006 who reported that in *Aspergillus* spp., peptone showed maximum cellulase activity. It is evident from the data (Table 17) that *Rhizopus* spp. exhibited maximum enzyme activity at 1% concentration of different nitrogen sources. Yeast extract proved to the best nitrogen source for the cellulase production (5.00 U/ml) followed by peptone (3.77 U/ml) and Beef extract (0.74 U/ml). More or less similar results were reported by Youssef and Berekaa, 2009 and Kashem *et al*., 2004 who observed that amongst different nitrogen sources tested, growth was boosted in presence of organic nitrogen source. It is observed from the data (Table 18) that enzyme activity by *Penicillium* spp. reached peak when different nitrogen sources were used at 1.5% concentration. When Beef extract was used as a sole nitrogen source the activity of cellulase was maximum (5.03 U/ml), whereas when peptone was used enzyme activity declined (3.99 U/ml). The present results are in accordance with Panagiotou *et al*., 2003 and Kachlishvili *et al*., 2006.

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