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Comparative Studies on the Amylase and Cellulase Production of *Aspergillus* and *Penicillium*

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ABSTRACT: Filamentous fungi are important due to their high enzymes production potential. Many enzymes produced by fungi have related to biotechnological applications in several industrial sectors. The purpose of this study was to collect and isolate *Penicillium* and *Aspergillus* species from different sources and examined for their ability to produce cellulase and amylase. Ten fungal isolates of genera, *Aspergilus* and *Penicillium* were examined for their ability to produce industrial important enzymes. All fungal isolates exhibited high DCZ/DFC ratio in cellulase and amylayes activity. It was also evident that starch and cellulose medium effected the fungal growth. Four *Penicillium* species viz. *P. janthinellum* (IK-48), *P. melinii* (IK-49) and *P. velutinum* (IK- 51) and *P. waskmanii* (IK- 50) showed the increased growth in starch and cellulose medium as compare to control. *P. waskmanii* (IK- 50) showed the highest growth stimulation in the cellulose and starch medium. This study contributes to catalogue local fungal isolated in Pakistan, and provides additional information to support future research about the industrial potential of these microorganisms for enzymes and, eventually, also secondary metabolites with anti-microbial or anti-parasitic activities. @JASEM

Keywords: Cellulose; Amylase; Fungi; Enzymes.

Several microorganisms can be obtained to enzyme production. However, enzymes from fungal and bacterial sources have dominated applications in industrial sectors. Filamentous fungi are important organisms for production of useful enzymes and biological active secondary metabolites. Studies on fungal enzymes especially in the developing countries are of great concern, probably because of the ubiquitous nature and non fastidious nutritional requirements of these organisms (Abe *et al*., 1988). *Aspergillus* and *Penicillium* are particularly interesting for industrial enzymes due to their easy cultivation, and high production of extracellular enzymes of large industrial potential. These enzymes are applied in many industries.

Amylases are important enzymes employed in the starch processing industries for the hydrolysis of polysaccharides such as starch into simple sugar constituents (Emmanuel *et al*., 2000; Pederson and Nielsen, 2000; Sarikaya *et al*., 2000) by degrading 1-4 linkage of starch. Besides their use in starch saccaharification, they also find potential application in a number of industrial processes such as in food, baking, brewing, detergent, textile and paper industries. With the advent of new frontiers in biotechnology, the spectrum of amylase application has expanded into many other fields, such as clinical, medical and analytical chemistry (Pandey *et al*., 2000).

Cellulases are hydrolytic enzymes capable of hydrolyzing the most abundant organic polymer i.e. cellulose to smaller sugar components. Cellulases have potential in industries and are used in food, beverages, textile, laundry, paper and pulp industries etc (Cavaco-Paulo and Gübitz, 2003; Jahangeer *et al*.,2005; Miettinen-Oinonen *et al*., 2004; Walsh, 2002). As lytic enzymes, they are of also major importance is the protoplast production (Bhat, 2000; Davis,1985; Mandels *et al*., 1974) for tissue culture and plant metabolites production.

In last two decade potential of using microorganisms as biotechnological sources of

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industrially relevant enzymes has stimulated interest in the exploration of extracellular enzymatic activity in several microorganisms (Abu *et al*., 2005; Akpan *et al*., 1999; Pandey *et al*., 2000). But still there is a need for explore new fungal isolates for amylases and cellulase activity to fulfill the industrial demand. Therefore, the present investigation deals with screening of local fungal isolates from different sources, for cellulase and amylase production for future investigation and industrial processes.

MATERIALS AND METHODS

*Isolation of Microorganism***:** Samples were collected from various locations and sources for *Penicilium* and *Aspergillus* species isolation. Dilution plate method and direct plate method were used for the isolation of fungal isolates (Table. 1).

Table 1. Sources of *Penicillium* and *Aspergillius* species

FCBP#	Fungal isolates	Sources
IK-48	*Penicillium janthinellum* [Biourge]	Rhizosphere of *Citrus limonia*
IK-49	*Penicillium melinii* [Thom]	Rhizosphere of *Citrus limonia*
IK-29	*Penicillium oxalicum* [Currie & Thom]	Rhizosphere of *Citrus limonia*
IK- 51	*Penicillium velutinum* [J.F.H. Beyma]	Rhizosphere of *Citrus limonia*
IK- 50	*Penicillium waskmanii* [K.M. Zalessky]	Rhizosphere of *Citrus limonia*
1121	*Aspergillus aculeatus*[Iizuka]	Guava rhizospheric soil
1048	*Aspergillus ficuum* [(Reichardt) Thom & Currie]	Paper mill effluent
0990	*Aspergillus japonicus* [Saito]	Shisham seed
1005	*Aspergillus niger* [Tiegh]	*Aloe vera* gel
1047	*Aspergillus phoenicis* [Cda.(Thom)]	Taxtile mill effluent

*Screening for Amylase and Cellulase Producing Fungal Strains***:** Amylase and cellulase screening was based on a plate culture method. For amylase screening plate medium contained (g/L): $NaNO_3$, 1.0; K_2HPO_4, 1.0; $MgSO_4$.$7H_2O$, 0.5; $FeSO_4$, soluble starch, 20; agar, 25. The initial pH was adjusted to 6.0. The medium was sterilized by autoclaving at 121 ^oC for 15 minutes. Fungi were plated on the agar medium and incubated at 28 ^oC for 7 days. Starch degrading activities were detected as clear zones after exposure to iodine solution. Diameters of the clear zones and fungal colonies were evaluated by millimetric ruler. Index of enzyme activity was recorded as clear zone ratios = clear zone diameter / colony diameter.

Cellulose activities of the fungal isolates were also determined by using plate screening medium (PSM) contained Mendel's mineral salt grams per litter (g/L) solution that is: Urea -0.3, $(NH_4)_2SO_4$ -1.4, KH_2PO_4- 2.0, $CaCl_2$- 0.3, $MgSO_4$ -0.3, yeast extract- 0.25 and proteose peptone -0.75 with 10 g L-1 of carboxymethyl cellulose(CMC) and 17.5 g L-1 agar (Mandels, 1974). Agar blocks (8 mm in diameter) from one-week old fungal colony grown on MEA plates were cut and inoculated in the centre of the basal media plates. The plates were incubated at 25$\pm$ 2 ºC for seven days.

Cellulose activities of the fungal isolates were also determined by using plate screening medium (PSM) contained Mendel's mineral salt grams per litter (g/L) solution that is: Urea -0.3, $(NH_4)_2SO_4$ -1.4, KH_2PO_4- 2.0, $CaCl_2$- 0.3, $MgSO_4$ -0.3, yeast extract- 0.25 and proteose peptone -0.75 with 10 g L-1 of carboxymethyl cellulose(CMC) and 17.5 g L-1 agar [11]. Agar blocks (8 mm in diameter)

from one-week old fungal colony grown on MEA plates were cut and inoculated in the centre of the basal media plates. The plates were incubated at 25± 2 ºC for seven days.

Cellulolytic fungal species were also identified on the basis of the diameter of the hydrolysis zone surrounding the fungal colonies. Fungal Plates were stained with 1% Congo red dye (30 min), followed by destaining with 1 M NaCl solution for 20 min. clear zones was observed only around colonies of the cellulolytic active fungal strains. Cellulose activity on carboxymethyl agar was recorded as the Index of Relative Enzyme Activity (ICMC) was recorded as clear zone ratios = clear zone diameter / colony diameter (Bradner *et al*., 1999; Pečiulytė, 2007;Teather and Wood,1982). Growth of fungal isolates on MEA was taken as control. Growth simulation/inhibition index was computed as the colony diameter on pectin agar/colony diameter on control agar ratio.

RESULTS AND DISCUSSION

Aspergillus and *Penicillium* strains were isolated from different sources (i.e. rhizospheric soil, fruits, seeds, Aloe gel and industrial effluent) and were identified on the basis of morphological characters (Table 1).

Screening for the production of amylase and cellulase by plate assays revealed the production of these enzymes by the formation of discolouration zones in agar plates. The ability of starch/ cellulose degrading activities of fungi was estimated in terms of diameter of clear zone (DCZ) /diameter of fungus colony (DFC) ratios. All fungal isolates exhibited high DCZ/DFC ratio in cellulase and amylayes activity (Table 2). However, zonation can not in any way be correlated quantitatively with the amount of enzyme produced. The results showed that all isolates possessed a high potential for amylase and cellulase production.

On the other hand, it was also evident that starch/ cellulose medium effected the fungal growth. Four *Penicillium* species viz. *P. janthinellum* (IK-48), *P. melinii* (IK-49) and *P. velutinum* (IK- 51) and *P. waskmanii* (IK- 50) showed the increased growth in starch and cellulose medium as compare to control. These strains were hyperactive in the presences of cellulose and starch. *P. waskmanii* (IK- 50) showed the highest growth stimulation in the cellulose and starch medium (Table 2). On the other hand, all *Aspergillus* species showed low growth stimulation on starch/ cellulose medium as compare to *Penicillum* species.

This may be attributed to their genetic makeup and subsequent physiological conditions (Parekh *et al*., 2000). It is enumerated from the spectrum of microbial cultures employed for amylase production in solid state fermentation that all isolates tested gave the highest detectable quantities of starch hydrolysis. These findings are in line with the work conducted by various workers (Omemu *et al*., 2005, Pandey *et al*.,2006, Sasi *et al*.,2010) where the selection of potent species was made by plate method. These results are also in agreement with the ones obtained by other workers, where residual enzyme activity was noted when cellulytic fungi were grown in presence of glucose and many fold increase in enzyme yield were reported in the presence of cellulosic substrate (Lederberg , 1992; Lynd *et al*., 2002). The production of cellulase for the utilization of cellulose is induced only in the presence of specific substrate (or product thereof) but suppressed when easily utilizable sugars such as glucose are available (Lynd *et al*., 2002). Although cellulases are inducible, but there is a low level of constitutive production of these enzymes suggesting that there may be isozymes, some of them remain repressed in absence of inducer and presence of inducer greatly affect the enzyme yield (Yalpani , 1987). However, cellulase production is also influenced by several other factors, such as carbon, nitrogen and phosphorus sources, the ratio of carbon to nitrogen provided, trace elements, pH and aeration rate (Philippidis , 1994). *Aspergillus*

species have been identified to possess all component of cellulase enzyme system (Vries and Visser, 2001) which is in agreement with the present study. *Penicillium* species with the ability to produce high cellulase and hemicellulase have been described (Brown *et al*., 1987), but much less is known about the regulation and production of these enzymes by *Penicillium* species compared to *Trichoderma* and *Aspergillus.*

Table 2. Comparative analysis of cellulase and amylase production by *Aspergillus* and *Penicillium* species.

Fungal isolates	CELLULASE					AMYLASE				
	A	B	*C*	D	E	F	G	H	I	J
Penicillium janthinellum	8.5± 0.09	8.5± 0.09	1.0± 0.01	4.4±0 .18	1.9 ±.02	9.0± 0.00	9.0± 0.00	1.0± 0.00	4.4± 0.18	2.0± 0.00
Penicillium melinii	7.3± 0.09	7.3± 0.09	1.0± 0.01	3.4±0 .15	2.1± 0.03	6.3± 0.23	6.3± 0.23	1.0± 0.04	3.4± 0.15	1.8± 0.07
Penicillium oxalicum	8.8± 0.09	8.8± 0.09	1.0± 0.01	6.1±0 .12	1.5± 0.02	9.0± 0.00	9.0± 0.00	1.0± 0.00	6.1± 0.12	1.5± 0.00
Penicillium velutinum	9.0± 0.00	9.0± 0.00	1.0± 0.00	3.7±0 .12	2.4± 0.00	8.4± 0.20	8.4± 0.20	1.0± 0.02	3.7± 0.12	2.2± 0.05
Penicillium waskmanii	9.0± 0.00	9.0± 0.00	1.0± 0.00	2.1±0 .06	3.2± 0.00	8.1± 0.06	8.1± 0.06	1.0± 0.01	2.1± 0.06	2.9± 0.01
Aspergillus aculeatus	7.5± 0.17	7.5± 0.17	1.0± 0.02	5.9±0 .09	1.3± 0.03	9.0± 0.00	9.0± 0.00	1.0± 0.00	5.9± 0.09	1.5± 0.00
Aspergillus ficuum	8.0± 0.06	8.0± 0.06	1.0± 0.00	6.1±0 .09	1.3± 0.01	9.0± 0.00	9.0± 0.00	1.0± 0.00	6.1± 0.09	1.5± 0.00
Aspergillus japonicus	7.3± 0.15	7.3± 0.15	1.0± 0.02	6.7±0 .12	1.1± 0.02	9.0± 0.00	9.0± 0.00	1.0± 0.00	6.7± 0.12	1.3± 0.00
Aspergillus niger	8.7± 0.09	8.7± 0.09	1.0± 0.01	7.7±0 .09	1.1± 0.01	9.0± 0.00	9.0± 0.00	1.0± 0.00	7.7± 0.09	1.2± 0.00
Aspergillus phoenix	7.3± 0.15	7.3± 0.15	1.0± 0.02	5.6± 0.10	1.3± 0.03	9.0± 0.00	9.0± 0.00	1.0± 0.00	5.6± 0.10	1.6± 0.00

A= Hydrolysis zone diameter [cm]; B= Colony diameter on CMC agar [cm]; C= Hydrolysis activity index (ICMC); D= Colony diameter on control agar [cm]; E= Growth stimulation/ inhibition index; F= Hydrolysis zone diameter [cm]; G= Colony diameter [cm] ; H= Hydrolysis activity index; I = Colony diameter on control agar; J= Growth stimulation/ inhibition index

On the basis of current status of modern biotechnology, amylases and cellulase are now gaining importance in industrial applications. Food and starch based industries is the major market and thus the demand of amylases would always be high in these sectors. There fore new sources of micorganism are needed to complete the industrial enzyme demand in future. It is concluded from present screening, further work is required to check the stability of enhanced production of these enzymes.

REFERENCES

Abe, J; Bergman, FW; Obeta, K; Hizukuri, S (1988). Appl. Microbiol. Biotechnol. 27, 447-450.

Abu, EA; Ado, SA; James, DB (2005). Afr. J. Biotechnol. 4, 785- 790.

Akpan, I; Bankole, MO; Adesemowo, AM; Latunde-Dada, GO (1999).Trop. Sci., 39, 77-79.

Bhat, MK (2000). Biotechnol Advances. 18, 355–383.

Bradner, JR; Gillings, M; Nevalainen, KMH (1999).World J. Microbiol. Biotechnol. 15, 131–132.

Brown, JA; Collin, SA; Wood, TM (1987). Enz. Microbial Technol. 9, 176–80.

Cavaco-Paulo, A; Gübitz, G (2003).Catalysis and processing. *In*: A. Cavaco-Paulo, G. Gübitz (ed.). Textile Processing with Enzymes. England, Woodhead Publishing Ltd: 86, 119.

Davis, B (1985). Factors influencing protoplast isolation. *In*: J. F. Peberdy, L. Ferenizy (eds.). Fungal Protoplasts: Applications in Biochemistry and Genetics (Marcel Dekker, New York,) p. 356.

Emmanuel, L; Stephan, J; Bernard, H; Abdel, B (2000). Enz. Microb. Technol. 26, 3-14.

Jahangeer, S; Khan, N; Jahangeer, S; Sohail, M; Shahzad, S; Ahmad, A; Khan, SA (2005). Pak. J. Bot. 37, 739-748.

Lederberg, J (1992). Cellulases. *In*: Encyclopaedia of Microbiology (Vol. 1; A-C) (Academic Press Inc.).

Lynd, LR; Weimer, PJ; van Zyl, WH; Pretorius, IS (2002). Micro. & Mol. Bio. Rev. 66, 506-577.

Mandels, ML; Hontz, L; Nistrom, J (1974).Biotechnol. Bioengineering 16, 471–493

Miettinen-Oinonen, A; Londesborough , J; Joutsjoki, V; Lantto R; Vehmaanperä, J (2004). Enz Microbial Technol. 34, 332–341.

Omemu, AM; Akpan, I; Bankole, MO; Teniola, OD (2005). Afr. J. Biotechnol. 4, 19-25

Pandey, A; Nigamp, VT; Socco, D; Singh, L; Mohan, R (2006). Biochem. 31, 35-152.

Pandey, A; Soccol, CR; Mitchell, D (2000). Process Biochem. 35, 1153-1169.

Parekh , S; VinciI, VA; Strobel, RJ (2000). Appl. Microbiol. Biotechnol. 54, 287- 301.

Pečiulytė, D (2007). Ekologija. 53, 11–18.

Pederson, H; Nielsen, J (2000). Appl. Microbiol. Biotechnol, 53, 278-281.

Philippidis, GP (1994). Enzymatic Conversion of Biomass for Fuel Production. (eds): M. E. Himmel *et al*., ACS symposium series 566.

Sarikaya, E; Higassa, T; Adachi, M; Mikami, B (2000). Proc. Biochem. 35, 711-715.

Sasi, A; Kani, M; Panneerselvam, A; Jegadeesh, G; Muthu, K; Kumar, MR (2010). Afr. J. Microbiol. Res. 4, 581-586.

Teather, RM; Wood, PJ (1982). Appl. Environm. Microbiol. 43, 777–780.

Vries RP; Visser, J (2001). Micro. Mol. Bio. Rev. 65, 497-522.

Walsh, G (2002). Proteins; Biochemistry and Biotechnology (John Wiley and Sons).

Yalpani, M (1987).Development and prospect in enzymatic biopolymer modification. *In*: Industrial Polysaccharide, Vol. 3 (Elsevier).