Isolation and screening of amylases, pectinases and cellulases producing fungi from organic wastes

Asmina Noureen[*], Shomaila Sikandar[†], Imran Afzal[‡], Sadaf Sarfraz[§]

Abstract

Filamentous fungi are ubiquitous in nature and produced wide range of industrially important extracellular enzymes. The main focus of the present study is the isolation of indigenous fungi capable of producing extracellular enzymes. In present study, total thirteen fungal isolates were isolated from different samples including; soil samples, organic waste, vegetables, dry fruits and tamarind bark. The fungal isolates were further screened for their amylolytic, cellulytic and pectinolytic activities on their selective media by using plate assay method. Among thirteen fungal isolates, four isolates gave maximum pectinolytic and cellulolytic activities, while only three fungal isolates gave highest amylolytic activity in qualitative analysis. Furthermore, potential fungal isolates were identified on the basis of colony morphology and microscopy. The identified fungal isolates were Cladosporium sp., Fusarium sp., Curvulari sp., and Trichoderma sp. The present study found huge applications of hydrolytic enzymes produced from indigenous fungi in different industries like food beverages, textile, paper and pulp.

Key words: Hydrolytic enzyme, Plate assay method, Indigenous fungi

Introduction

Hydrolytic enzymes are catabolic enzymes that break the chemical bond between atoms of large molecule in the presence of water. Filamentous fungi are major source of hydrolytic enzymes due to their enhanced capacity for extracellular protein production (Kanimozhi and Nagalakshmi, 2014). Cellulose is linear polysaccharide carbohydrate with 1000-1200 glucose residue with β-1,4 glycosidic linkages. It is degraded by an enzyme called cellulase which are synthesized by bacteria and fungi during growth on cellulosic material (Kuhad, 2011). Fungi such as *Trichoderma spp, Penicillium spp, Aspergillus spp.* have ability to produce cellulytic enzymes from organic waste and soil (Naveenkumar, 2013). Cellulase have various applications and biotechnological potential for many industries (Howard, 2003). Now days, cellulases account for

[*]Department of Biology, Lahore Garrison University, Lahore, Pakistan
asminanoureen50@gmail.com
†Department of Biology, Lahore Garrison University, Lahore, Pakistan
Shomailasikandar11@gmail.com
‡Department of Biology, Lahore Garrison University, Lahore, Pakistan
imranafzal@lgu.edu.pk
§ Department of Chemistry, Lahore Garrison University, Lahore, Pakistan
dr.sadafsarfraz@lgu.edu.pk

approximately 20% of the world enzymes market used on industrial scale (Maheswari, 2000).

Amylase enzyme are glycoside hydrolases and act on α-1, 4-glycosidic. *Aspergillus niger* is used for the commercial production of amylases because they are ubiquitous in nature (Abu et al., 2005; Sunder et al., 2012). Fungal amylases found huge application in starch processing industries and in the formulation of enzymatic detergents (Suganthi et al., 2011). Pectin, a structural heteropolysaccharide naturally occurring biopolymer with complex fibers (Geetha et al., 2012; Gummadi and Panda, 2003). Apple and citrus fruits are the major sources of pectin (Reddy and Sreeramulu, 2012). Fungal pectinases used in several industrial processes, such as in food and beverages, plant fiber processing, tea, coffee, and oil extraction and for the treatment of pectinacious industrial wastewater (Reddy, 2012). Hydrolytic enzymes from fungal sources have gained much attention because they are the natural degraders and used as an alternative for production of industrial enzyme (De Castro et al., 2010; Varalakshami et al., 2008). Different approaches were used for the screening of fungal hydrolytic enzymes among them qualitative assays are powerful tools used for the screening of starch, pectin and cellulose degrading fungal enzymes. Qualitative results provide data on the basis of zone of inhibition which is useful for the screening of large number of isolates and several classes of enzymes (Kudah et al., 2011). The present study was used to isolate indigenous fungal isolates using different organic samples and also identify the potential pectinase, amylase and cellulase producing fungi through conventional methods.

Methodology

Collection of samples

For the isolation of potential fungi producing hydrolytic enzymes, organic wastes including; vegetables, ginger, green chilli, tomato, lemon, almond, walnut, peanuts and teramand bark were collected from local market of Lahore. While, soil samples were collected in polythene bags under sterile conditions from botanical garden in Lahore Garrison University, DHA, Lahore, respectively.

Isolation of fungi

Potato dextrose agar (PDA) plates were prepared for the isolation of fungi. All the collected samples were centrally inoculated on PDA plates, while, serial dilution and spread plate method was used for soil samples. All plates were incubated at 30^{o}C for 72 hours for fungal growth. After incubation, fungi growing on plates were further purified by sub-culturing to get pure cultures of fungi. Purified fungi were maintained on

PDA slants for further experimentations.

Screening of pectinases

For the screening of pectinases, following media with some modification was used 0.05g $MgSO_4$, 1.0g K_2HPO_4, 1.0g $NaNO_3$, 0.01g $FeSO_4$, 20.0g Pectin, and 20.0g agar to 1 L of distilled water to screen for pectinolytic activity. Amoxicillin was added to prevent bacterial growth. All the fungal isolates were inoculated on pectin screening media and incubated at 30ºC for 72 hours. After incubation, the plates were stained with 50 mM iodine solution to view the clear zones around the fungal colonies.

Screening of cellulases

For the screening of fungal cellulases, all fungal isolates were inoculated on petri plates containing media with carboxymethyl cellulose (CMC) and incubated at 30ºC for 72 hours of incubation, after which they were stained with Congo Red dye to visualize clearance zones after the duration of 15 min.

Screening for amylase

For the screening of amylases, fungal isolates were inoculated on media containing PDA with starch. Amoxicillin was added to avoid bacterial contamination. Plates were incubated at 30ºC for 72 hours. After incubation, stained the plates with 50 Mm iodine solution for 15 min for the visualization of clear zones.

Results

A total of 13 fungal isolates isolated from organic wastes and soil. Isolated fungal colonies were maintained on PDA and named as BL1, BL2, BL3, BL4, BL5, BL6, BL7, BL8, BL9, BL10, BL11, BL12 and BL13, respectively (Fig.1).

Screening of cellulase producing fungi

Fungal isolates were tested for cellulase production by using CMC media. Production of cellulase was indicated by zone of clearance around the fungal growth (Table 1). Among all the isolates, following showed maximum zone of hydrolysis i.e., BL3, BL5, BL7 and BL,11, respectively as shown in figures 2-5.

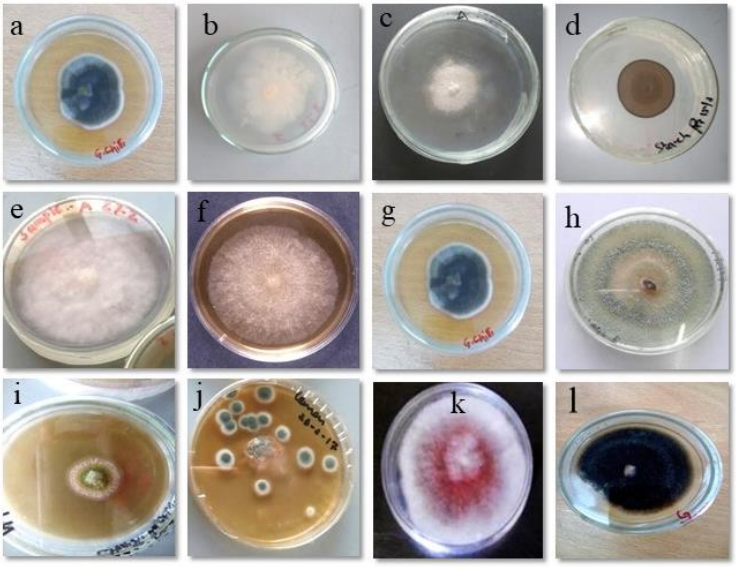

Figure 1: Isolated fungal morphology on PDA plates
(a;BL1, b;BL2, c;BL3, d;BL4, e;BL5, f;BL6, g;BL7, h;BL8, i;BL9, j;BL10, k;BL11, l; BL12)

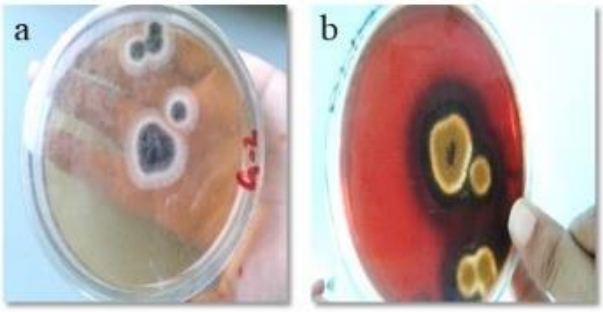

Figure 2: Cellulase production in terms of zone of clearance around fungal isolate BL3 (a) Growth before screening. b) Zone of clearance after screening

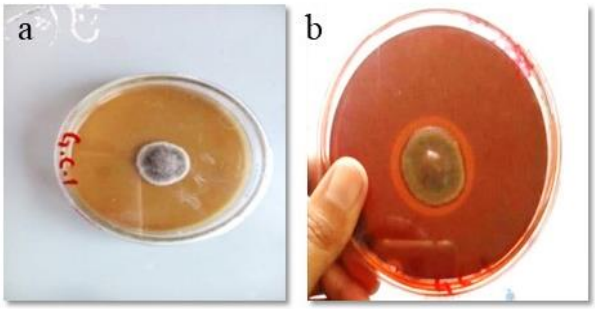

Figure 3: Cellulase production in terms of zone of clearance around fungal isolate BL5 (a) Growth before screening. b) Zone of clearance after screening

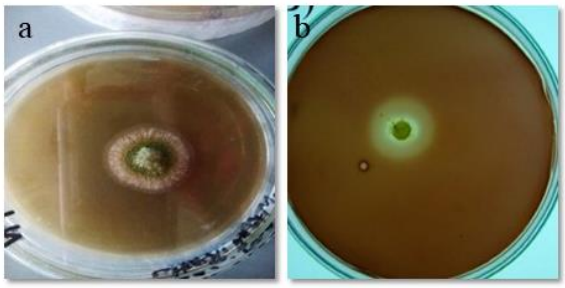

Figure 4: Cellulase production in terms of zone of clearance around fungal isolate BL7 (a) Growth before screening. b) Zone of clearance after screening

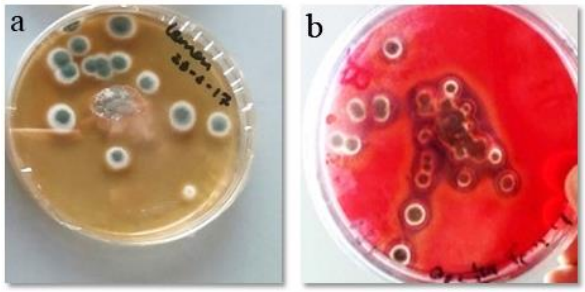

Figure 5: Cellulase production in terms of zone of clearance around fungal isolate BL11 (a) Growth before screening. b) Zone of clearance after screening

Table 1

Measurements of zones of hydrolysis for the production of cellulases

Fungal ID	Zones before screening (mm)	Zones after screening (mm)	Relative diameter of zones (mm)
BL3	4	4.5	1.12
BL5	3.1	3.2	1.03
BL7	2	3.6	1.8
BL11	3.9	4.2	1.07

Screening of amylase producing fungi

Fungal isolates were tested for the production of amylases by using starch hydrolysis test. Among all the fungal isolates screened qualitatively, BL2, BL5 and BL8 gave maximum amylase production in terms of zone of hydrolysis Table 2, (Fig. 6-8).

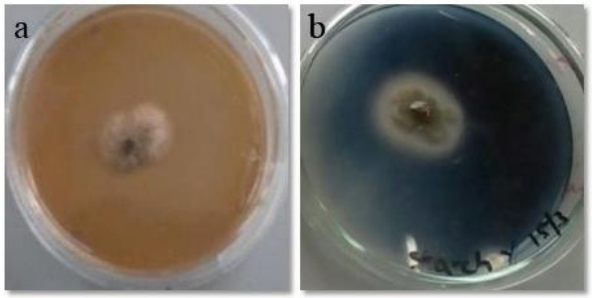

Figure 6: Amylase production in terms of zone of clearance around fungal isolate BL2 (a) Growth before screening. b) Zone of clearance after screening

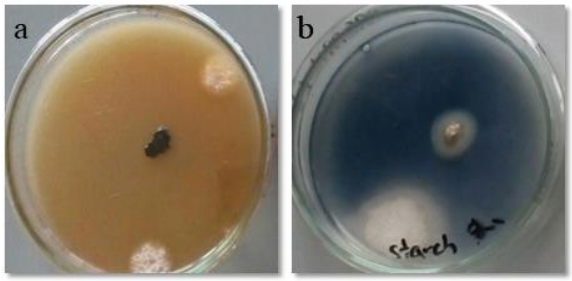

Figure 7: Amylase production in terms of zone of clearance around fungal isolate BL5 (a) Growth before screening. b) Zone of clearance after screening

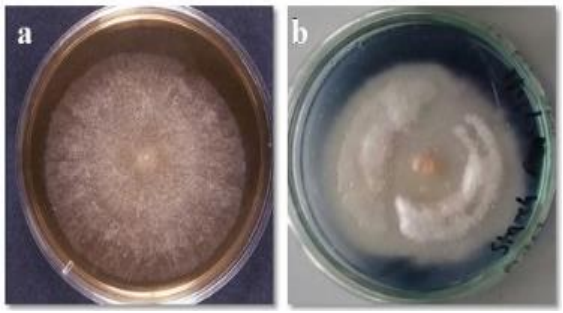

Figure 8: Amylase production in terms of zone of clearance around fungal isolate BL8 (a) Growth before screening. b) Zone of clearance after screening

Table 2
Measurements of zones of hydrolysis for the production of amylases

Fungal ID	***Zones before screening (mm)***	***Zones after screening (mm)***	***Relative diameter of zones (mm)***
BL2	1.8	2.3	1.27
BL5	6.5	8	1.23
BL8	3	3.5	1.16

Pectinase screening

Fungal isolates were used for the qualitative screening of

pectinases. Among thirteen fungal isolates, four gave maximum pectinase production in terms of zone of hydrolysis including BL4, BL5, BL6 and BL11 showed in figures 9-12 and Table 3.

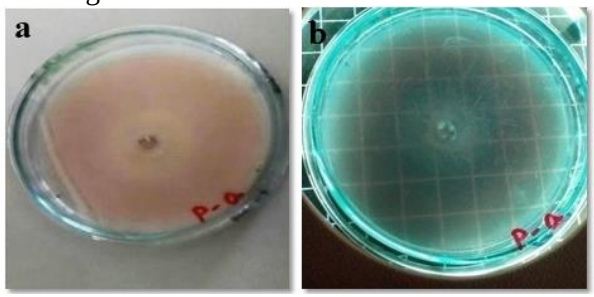

Figure 9: Pectinase production in terms of zone of clearance around fungal isolate BL4 (a) Growth before screening. b) Zone of clearance after screening

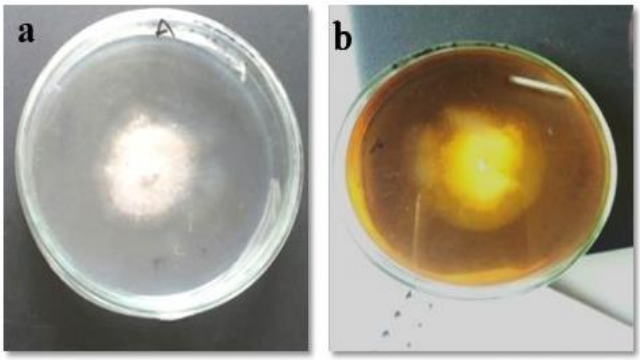

Figure 10: Pectinase production in terms of zone of clearance around fungal isolate BL11 (a) Growth before screening. b) Zone of clearance after screening

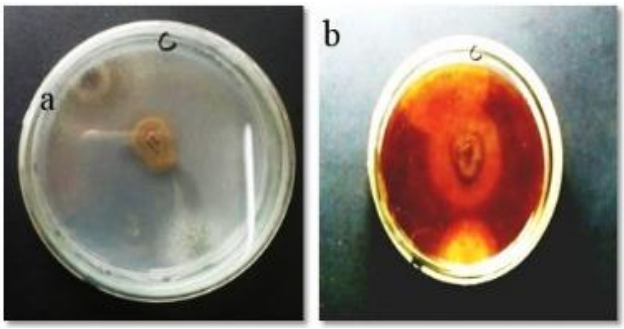

Figure 11: Pectinase production in terms of zone of clearance around fungal isolate BL5 (a) Growth before screening. b) Zone of clearance after screening

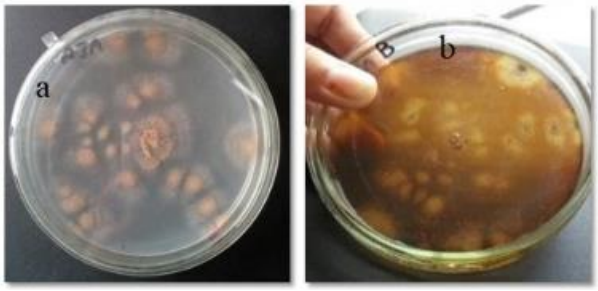

Figure 12: Pectinase production in terms of zone of clearance around fungal isolate BL6 (a) Growth before screening. b) Zone of clearance after screening

Table 3
Measurements of zones of hydrolysis for the production of pectinases

Fungal ID	Zones before screening (mm)	Zones after screening (mm)	Relative diameter of zones (mm)
BL4	3.5	3.7	1.05
BL5	2.5	4.2	1.68
BL6	3.8	4.5	1.18
BL11	4	4.4	1.1

Microscopy

Potential fungal isolates BL3, BL5, BL8 and BL11 were further selected for their microscopic identification. These four fungal isolates were microscopically identified using lacto-phenol blue dye. Fungal isolate BL11 resembles to *Fusarium sp.,* because of macroconidia observed randomly under the microscope. BL8 fungal isolate resembles to *Trichoderma sp.,* because conidia and conidiophore were observed under the microscope. However, fungal isolate BL3 closely identified to *Curvularia sp.,* due to four cellular, curved conidia were present in sympodial mode. While, BL5 fungal isolate resembles to *Cladosporium sp.,* because dark pigmented conidia were observed in simple and branching form.

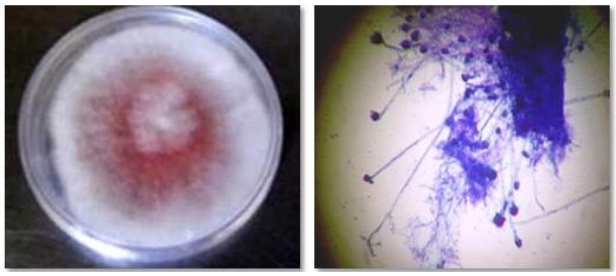

Figure 13: Microscopic identification of Fusarium sp., BL11

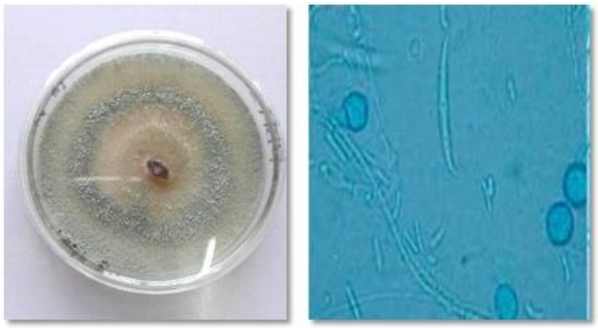

Figure 14: Microscopic identification of Trichoderma sp., BL8

Figure 15: Microscopic identification of Curvulari sp.,BL3

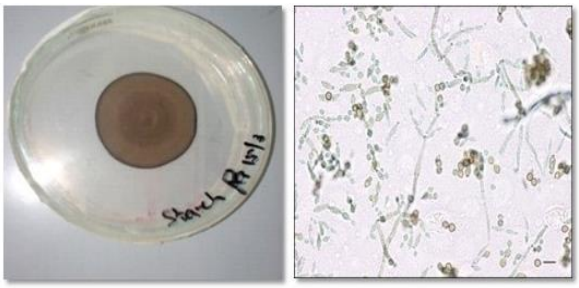

Figure 16: Microscopic identification of Cladosporium sp.,BL5

Discussion

Main purpose of the study is to screen fungal pectinolytic, cellulytic and amylolytic enzymes from the cheap sources i.e., fruits, vegetables and soil. In present study, fungal isolates including *Trichoderma sp., Penicillium sp., Aspergillus sp., Cladosporium sp.,* and *Fusarium sp.,* were isolated from different sources of tomato, chilli and soil. Recent studies also showed that rotten tomato, chilli and soil are the main sources of *Penicillium citrinum, Trichoderma sp.,* and *Aspergillus sp.,* (Chigoziri and Ekefan 2013; Elisane et al., 2008; Kolte and Spakal 1994; Kostadinovaa et al., 2014; Sharma 2010; Sandyha, 2013; Reddy et al., 2014). The fungal isolate *Curvularia sp.,* in the present study was

isolated from garlic source and the work coincided with the Gautam et al, in 2014, isolated *Curvularia lunuta* from garlic source.

Microbial pectinase are the most significant enzymes used in fruit juice industry. Fungal pectinase is of industrial importance and have been exploited commercially. Out of thirteen isolates, five isolates showed pectinolytic activity in terms of clear zone of hydrolysis around the growth. The fungal isolates showed highest production of pectinases in *Fusarium sp.* BL11, *Trichoderma sp.* BL8, and *Cladosporium sp.* BL5, respectively. Similar studies showed the production of pectinase under liquid state fermentation conditions (Sandyha, 2013; Reddy 2012).

Amylase is significant enzymes employed in starch processing industries for the hydrolysis of polysaccharides. Microbial amylases meet industrial demands. Enzymes from fungal sources have dominated use in industrial field. In fungal sources *Aspergillus spp* and *Penicillium spp* are the most important amylase producers (Ibatsamet al., 2011). Out of six fungal cultures, only three gave positive results of amylase production including; *Fusarium sp.* BL11, *Aspergillus sp.*, and *Penicillium sp.* Similar results have been reported by Forgarty and Kelly 1979. In the present work, there were significant results of fungal cellulose production. Samples were taken from soil, green chili and garlic. Out of six isolates, four isolates showed positive results including; *Trichoderma sp.*, *Fusarium sp.*, *Curvularia sp.*, and *Cladosporium sp.* Recent investigations revealed that fungi such as *Trichoderma sp.*, and *Fusarium sp.*, have ability to produce cellulytic enzymes from organic waste and soil (Baldrian and Gabriel, 2003; Gomes et al., 2006; Naveenkumar, 2013).

Conclusion

In this study, *Trichoderma* and *Fusarium* had a higher relative rate of occurrence in the organic waste and soil. The isolated fungi also produced cocktail of cellulases, pectinases and amylases which will be further studied for their enzymatic potentials in the industrial applications and in bioconversion of lignocellulosic waste to valuable products. Fungi which are less time consuming and prompt production have replaced other chemicals for enzyme manufacture in industrial use.

References

Baldrian, P., & Gabriel, J. (2003). Lignocellulose degradation by Pleurotusostreatus in the presence of cadmium. FEMS *Microbiology letters*, 220(2), 235-240.

Chigoziri, E., &Ekefan, E. J. (2013). Seed-borne fungi of Chilli pepper

(Capsicum frutescens) from pepper producing areas of Benue state, Nigeria. *Agriculture and Biology Journal of America*, 4, 370-374.

de Castro, A. M., Ferreira, M. C., da Cruz, J. C., Pedro, K. C. N. R., Carvalho, D. F., Leite, S. G. F., & Pereira, N. (2010). High-yield endoglucanase production by *Trichoderma harzianum* IOC-3844 cultivated in pretreated sugarcane mill byproduct. *Enzyme research*, 2010.

Gautam, S. P., Bundela, P. S., Pandey, A. K., Khan, J., Awasthi, M. K., &Sarsaiya, S. (2011). Optimization for the production of cellulase enzyme from municipal solid waste residue by two novel cellulolytic fungi. *Biotechnology research international*, 2011.

Gautam, S., Bundela, P., Pandey, A., Awasthi, M., & Sarsaiya, S. (2012). Diversity of cellulolytic microbes and the biodegradation of municipal solid waste by a potential strain. *International Journal of Microbiology*, 2012.

Geetha M, Saranraj P, Mahalakshmi S, Reetha D. (2012). Screening of pectinase producing bacteria and fungi for its pectin hydroolytic activity using fruit wastes. *Int J Biochem Biotech Sci*, 1: 30-42.

Gummadi, S. N., & Panda, T. (2003). Purification and biochemical properties of microbial pectinases—a review. *Process Biochemistry*, 38(7), 987-996.

Howard, R., Abotsi, E., Van Rensburg, E. J., & Howard, S. (2003). Lignocellulose biotechnology: issues of bioconversion and enzyme production. *African Journal of Biotechnology*, 2(12), 602-619.

Kanimozhi, K., Nagalakshmi, P. K. (2014). Xylanase production from *Aspergillus niger* by solid state fermentation using agricultural waste as substrate. *International Journal of Current Microbiology and Applied Sciences*, 3(3), 437-446.

Kuhad, R. C., Gupta, R., & Singh, A. (2011). Microbial cellulases and their industrial applications. *Enzyme research*, 2011.

Naveen Kumar, K., & Thippeswamy, B. (2013). Isolation and screening of potential cellulolytic fungi from Areca nut husk waste. *Int. J.*

Curr. Sci, 8, 125-132.

Nigam, P., & Singh, D. (1995). Enzyme and microbial systems involved in starch processing. *Enzyme and Microbial Technology*, 17(9), 770-778.

Pandey, A., Selvakumar, P., Soccol, C. R., & Nigam, P. (1999). Solid state fermentation for the production of industrial enzymes. *Current science*, *77*(1), 149-162.

Reddy, P. L., & Sreeramulu, A. (2012). Isolation, identification and screening of pectinilytic fungi from different soil samples of Chittoor district. *International Journal of Life Sciences Biotechnology and Pharma Research*, 1(3), 1-10.

Singh, S., Moholkar, V. S., & Goyal, A. (2013). Isolation, identification, and characterization of a cellulolytic *Bacillus amyloliquefaciens* strain SS35 from rhinoceros' dung. ISRN microbiology, 2013.

Souza, P. M. d. (2010). Application of microbial α-amylase in industry-A review. *Brazilian Journal of Microbiology*, 41(4), 850-861.

Suganthi, R., Benazir, J., Santhi, R., Ramesh Kumar, V., Anjana Hari, N. M., Nidhiya, K., . . . Lakshmi, R. (2011). Amylase production by *Aspergillus niger* under solid state fermentation using agro industrial wastes. *International Journal of Engineering Science and Technology, 3(2), 1756-1763.*

Sun, Y., & Cheng, J. (2002). Hydrolysis of lignocellulosic materials for ethanol production: a review. *Bioresource technology*, 83(1), 1-11.

Sundar, R., Liji, T., Rajila, C., & Suganyadevi, P. (2012). Amylase production by *Aspergillus niger* under submerged fermentation using ipomoea batatas. *Int J Appl Biol Pharmac Technol*, 2012; 3(1): 175-182.