Production of Enzymes in Predominant Thermophilic Fungi Available From Organic Substrates

G.M.Birajdar, and U.N.Bhale*

*Research Laboratory, Department of Botany, Arts, Science and Commerce College, Naldurg, Tq. Tuljapur, Dist Osmanabad, 413602 (M.S.) India

Abstract

Present investigation describes that the study site comes under Aurangabad Division Maharashtra and it falls in Deccan Plateau Zone of India. It was collected different types of organic substrates viz. vermiompost, poultry manure, baggase, farm yard manure (FYM), soil, Ash etc. Isolated thermophilic predominant fungi thermophilic fungi viz. Aspergillus niger, Mucor mucedo, Humicola insolens, Trichoderma harzianum, T. viride, Penicillium duponti, Fusarium oxysporum and Chaetomium thermophilum were carried out for the production of enzymes. Isolated predominant thermophilic fungi were evaluated on different types of enzymes. Among tested thermophilic fungi, the highest activity was observed in C. thermophilium (20mm) followed by T. harzianum (19.50mm) In lipase, M. mucedo (15.40mm) was found maximum followed by F. oxysporum. Cellulase activity was found highest in A. nige (25mm) followed by others. In case of xylanase, catalase, peroxidase and esterase activities were found maximum, minimum and medium even negative in some fungi. Maximum pectinase activity was detected from H. insolens (52.26 @ 0 min) and (74.25 @ 10 min) and in case of M. mucedo, F. oxysporum and C. thermophilium was found most extreme while least in A. niger (30.12) and P. duponti (33.47) @ 0 minute.

Key words: Organic Substrates, Thermophilic Fungi, Enzymes

I Introduction

Microorganisms play a very important role from the groups according to their temperature ranges i.e. psychrophiles, mesophiles and thermophiles. Of the 70,000 formally recorded fungi species only around 30 species have the ability to grow at these elevated temperatures. Temperature is one of the extremely important environmental variables that play a decisive role in the survival, growth, distribution and diversity of microorganisms on the surface of the earth. The response of fungi to temperature varies between the two extremes of obligatorily thermophilic through thermostolerance to psychrophilic species. However, by far the majority of known fungi are mesophiles developing in culture between 5 and 37°C; the psychrophiles extend below that range of temperatures (Dix and Webster, 1995). (Atkinson, 1997) suggested that once temperature goes beyond 40°C, the mesophilic microbes become less aggressive and are replaced by thermophilic microbes, during thermophilic stage high temperatures accelerate up to 65°C, the breakdown of organic and inorganic compounds like cellulose and hemicelluloses, the major structural molecules. Through microbial decomposition of the organic waste matter which can be stabilized, matured and deodorized in to a product rich in humic substances that can be used as organic soil conditioner which is easy to store and distribute (Sahu et al., 2015).

Thermophilic aerobic micro-organisms are physiologically very active and are capable of producing several thermostable enzymes responsible for decomposition of cellulose and to a great extent lignin into simpler compounds. Thermophilic fungi are the chief components of the microflora that develops in heaped masses of plant material, piles of agricultural and forestry products and other accumulations of organic matter wherein the warm, humid, and aerobic environment provides the basic conditions for their development (Allen et al., 1949). Furthermore, compost has a high nutritional value with high concentrations of especially
nitrogen, phosphorus and potassium, while the contamination by heavy metals and other toxic substances are very low (Asghar et al., 2006).

Naik (2017) describes the concepts of compost i.e. organic matter is an important component of soil which includes of plant and animal residues that are made up of complex carbohydrates, starch, cellulose, hemicellulose, lignin, protein, fats, organic acids, oils resins etc. Agricultural and industrial wastes were includes rice bran, rice husk, rice straw, sugarcane trash, bagasse, molasses, press mud etc., have a huge potential for recycling nutrient elements. The animal wastes such as cattle dung, buffalo manure, poultry wastes, rural and urban organic wastes, municipal waste can also be used for bioconversion as organic manure. Degradation is carried out by huge mixture of bacteria, fungi, insects, worms and other organisms that eat materials and recycle them into new forms (Singleton and Sambury, 1998).

Several thermophiles were observed to grow on starch, cellulose, hemicellulose, lignocellulose, lignin and pectin, but their ability to degrade lignin is doubtful (Johri et al., 1999). It was reported previously that the thermophilic fungi are known to produce thermotolerant proteases, lipases, amylases, cellulases, xylanases, lactases, trehalases and other extracellular enzymes (Johri et al. 1985; Satyanarayana et al., 1992).

According to Bending and Read (1997) the lactase production ability of the microorganisms has not been measured in the selected thermophiles with high polyphenol oxidase activity, but phenol oxidase producing ability of the isolates could accelerate the degradation of lignin. However, cellulose recalcitrance to biodegradation poses several major bottlenecks in the thermophilic digestion of biomass with the major impediment being the lack of availability of robust cellulases that can function efficiently at relatively higher temperatures (Rastogi et al., 2010).

In literature, many thermophilic celluolytic fungal species such as Sporotrichum thermophile, Thermoascus aurantiacus and Thielavia terrestris have been reported (Maheshwari et al. 2000). Thermophilic microbes growing at temperature of 50-80°C are the sources of highly active and thermostable enzymes studied (Haki and Rakshit 2003, Viikari et al., 2007,Yeoman et al., 2010, Zambare et al., 2011).

Similarly, during the production of lipase by Aspergillus sp. (Cihangir and Sarikaya, 2004) and Penicillium restrictum (De Azeredo et al.,2007) the highest lipolytic activity has been attained in media containing olive oil, Thermus thermophilus, whereas the optimum lipase production was attained at 70°C (Domínguez, 2005). Joshi et.al. (2008) reported the alkaline proteases, chitinases, amylases, lipases and caseinases enzymes in a wide range of microorganisms isolated from Soda Lake environments, such as Rift valley Soda lakes. Other lignolytic microorganism reported from Aspergillus flavus (Betts and Dart, 1988), Trichoderma harzianum (Harper and Lynch,1985; Bhale and Rajkonda, 2012), Nocardia sp. (Trojanowski et al., 1977)

II Materials and Methods
Survey of various compost

Study site (Osmanabad district) is comes under Marathwada regions of Maharashtra state of India. The region comes under Aurangabad Division. It was a part of Nizam’s domain, which was known as ‘The Princely State of Hyderabad’. This region lies between 17° 35’ N & 20° 40’ N Latitude and 70° 40’ E & 78° 15’ E Longitude. It falls in Deccan Plateau Zone of India with geographical area of 6.5 million hectare occupying 21 % of total area of the Maharashtra. This region is situated at an average height of about 300-650 m. above mean sea level, gradually sloping ranges originating from the Sahyadri’s in the west and Satpuda ranges in the north.

Isolation of thermophilic fungi by Dilution plate technique

The isolation of thermophilic fungi from different substrates was carried out using dilution plate technique (Apinis 1963; Waksman, 1939). Ten grams of sample were transferred to a flask containing 100 ml sterile water. The contents were shaken with centrifuge machine for 15 min and then diluted 10^{-3} of 0.5 ml was transferred to sterile petri plates containing different media in triplicates. The pH of medium was adjusted to 6.5 with 0.1N HCl or 0.1N NaOH. Petri plates were incubated in an inverted position at room temperature (RT) and adjusted the temperature in hot air oven at 35 to 65 °C. Pure cultures of isolates were maintained on respective media slants at 40°C for further study.
Identification of thermophilic fungi
The different topographical characters of the colonies were recorded at regular time intervals. The semi-permanent slides of the isolated fungi were prepared using 1% cotton blue and lactophenol. Identification of thermophilic fungi was made by referring relevant literature and monographs (Subramanian, 1971; Barnett, 1972; Kumar et al., 2010). Key to the identification of thermophilic fungi was used according to Salar and Aneja (2007).

Enzymes production of predominant thermophilic Fungi
Detection of enzyme activities of isolated thermophilic fungi from various composts was tested. Hydrolytic enzymes, cellulase, amylase, lipase and pectinase, which provide the fungi chemical means of entrance into the host and a process whereby nutrients, can be digested. The extracellular production of hydrolytic enzymes was important activity with respect to mycoparasitism and antibiosis of the fungal species. Commercial production of extracellular hydrolytic enzymes was employed commercially and the work about the enzyme activities is still going progressively. Cellulose & Lipase production activity was determined by Cup plate method (Dingle et al., 1953). The amylase activity was determined with the help of cup method (Singh and Saxena, 1982; Hankin and Anagnostakis, 1975). The enzyme assay for pectinolytic enzyme was tested by viscometer method (Papdiwal, 1982). Determination of xylanase activity was performed by Nakamura et al. (1993) Determination of Catalase and Peroxidase activity was performed by (Balasundaran, 2008). Determination of esterase activity was described by Sierra (1957).

III Results and Discussion
Isolation and Identification of thermophilic fungi
Study sites shows different types of available compost viz. vermiompost, poultry manure, baggase, farm yard manure (FYM), soil, Ash etc. Isolation of fungi were separated from substrate and among six substrates and isolated predominant thermophilic fungi viz. Aspergillus niger, Mucor mucedo, Humicola insolens, Trichoderma harzianum, Trichoderma viride, Penicillium duponti, Fusarium oxysporum and Chaetomium thermophilum

Enzyme Production
Isolated predominant thermophilic fungi were evaluated on different types of enzymes. In case of amylase, the fungi i.e. Aspergillus niger, Mucor mucedo, Humicola insolens and Chaetomium thermophilum were found most extreme activity while Penicillium duponti and Fusarium oxysporum was least activity. In lipase, A. niger, H. insolens, F. oxysporum and C. thermophilium was found least activity while there was negative activity found in Trichoderma harzianum and Penicillium duponti. In cellulase, A. niger, T. harzianum and P. duponti, were found most extreme activity while F. oxysporum was least. In cellulase there was negative activity in case of M. mucedo, H.insolens and Chaetomium thermophilum. In xylanase, A. niger, H. insolens, P. duponti and C. thermophilium found least while T. harzianum was most extreme activity. In addition there was negative activity in M.mucedo and F. oxysporum. In catalase, A. niger, F. oxysporum and Chaetomium was least while there was negative activity in H. insolens and T. harzianum. In peroxidase, A. niger, T. harzianum and P. duponti were found medium activity and there was negative activity in case of M. mucedo, F. oxysporum and C. thermophilium species. In esterase, there was negative activity in A. niger, M.mucedo and T. harzianum while P. duponti was found least (Tab.1, Fig.1).
Table 1. Detection of different enzymes of predominant thermophilic fungi.

| Predominant thermophilic Fungi | Enzyme Activity |
|--------------------------------|-----------------|
|                               | Amylase (mm) | Lipase (mm) | Cellulase (mm) | Xylanase | Catalase | Peroxidase | Esterase |
| Aspergillus niger             | 17.00        | 12.00       | 25.00          | +        | +        | ++         | -        |
| Mucor mucedo                  | 15.60        | 15.40       | 00.00          | -        | ++       | -          | -        |
| Humicola insolens             | 0.00         | 11.00       | 00.00          | +        | -        | +          | ++       |
| Trichoderma harzianum         | 19.50        | 00.00       | 19.00          | +++      | -        | ++         | -        |
| Trichoderma viride            | 00.00        | 12.06       | 15.32          | +++      | ++       | -          | +        |
| Penicillium duponti           | 13.90        | 00.00       | 20.00          | +        | ++       | ++         | +        |
| Fusarium oxysporum            | 14.50        | 14.50       | 15.00          | -        | +        | -          | -        |
| Chaetomium thermophilum       | 20.00        | 13.00       | 00.00          | +        | +        | -          | ++       |
Fig.1. Enzyme Production Of Predominant Fungi (1-Xylanase activity (A.niger), 2- Xylanase Activity (T.harzianum), 3-Amylase Activity (P.dupanti), 4- Amylase Activity (C.thermophylium), 5- Cellulase Activity(T. viride), 6- Cellulase Activity (P.dupanti), 7-Catalase activity (A.niger), 8- Catalase activity (T. viride), 9- Peroxidase activity(A.niger)

Pectinase Enzyme
Detection of another extracellular hydrolytic enzyme, pectinase was also undertaken in the present work. The pectinase activity was determined in terms of percent loss of viscosity by viscometry method. The percent loss of viscosity was obtained at a particular time intervals (Tab. 2). It was cleared from the results that loss of viscosity was directly related with the time. As time interval increased more viscosity loss was observed. Pectinase activity was detected from Aspergillus niger (30.12@0 min.) and (55.25 @10 min.). Maximum pectinase activity was detected from Humicola insolens (52.26 at 0 min) and (74.25 @ 10 min). In case of Mucor mucedo, Fusarium oxysporum and C. thermophilum was found most extreme while least in A. niger (30.12) and Penicillium duponti (33.47) @ 0 minute.

Table 2. Detection of pectinase enzymes from predominant thermophilic fungi.

G.M.Birajdar, IJSRM Volume 09 Issue 11 November 2021 [www.ijsrm.in]
| Sr. No. | Predominant thermophilic Fungi            | % Viscosity loss after time (Minutes) |
|--------|------------------------------------------|--------------------------------------|
|        |                                          | 0         | 01        | 05         | 10         |
| 1      | Aspergillus niger                        | 30.12     | 42.52     | 49.12      | 55.25      |
| 2      | Mucor mucedo                             | 48.21     | 53.42     | 58.97      | 64.32      |
| 3      | Humicola insolens                       | 52.26     | 59.25     | 67.24      | 74.25      |
| 4      | Trichoderma harzianum                   | 44.27     | 45.54     | 50.82      | 52.73      |
| 5      | Trichoderma viride                      | 43.85     | 46.22     | 50.42      | 53.06      |
| 6      | Penicillium duponti,                    | 33.47     | 46.58     | 52.12      | 58.54      |
| 7      | Fusarium oxysporum                      | 40.33     | 54.69     | 61.59      | 67.56      |
| 8      | Chaetomium thermophilum                 | 48.84     | 57.72     | 63.84      | 67.63      |

Study discussed here the different types of enzymes production by dominant thermophiles fungi involved in composts was detected and observed very promising role by these fungi for decomposition of composts and found passive and negative enzyme activity.

In literature, thermophilic cellulolytic fungal species such as *Sporotrichum thermophile*, *Thermoascus aurantiacus* and *Thielavia terrestris* have been reported and shows enzyme systems produced by various cellulolytic microorganisms for the degradation of cellulose and xylan (Aro et al., 2005). In comparison to mesophilic fungi, thermophilic ones have been found to show rapid growth rates and higher rates of cellulose decomposition, making thermophilic fungi an attractive potential source of cellulases (Rajasekaran and Maheshwari, 1990, Bhalla et al., 2013). Lee et al (2014) reported three species of thermophiles were isolated from compost and were identified as *Myriococcum thermophilum*, *Thermoascus aurantiacus*, and *Thermomyces lanuginosus* and grow at temperatures above 50°C and produce high levels of cellulolytic and xylanolytic enzymes at high temperatures. Msaraha et al. (2018) reported eight thermophilic bacteria were isolated and determined to have at least three strong enzyme activity including protease, lipase, amylase, cellulase, pectinase and xylanase. Bairagi (2016) reported total 151 fungal isolates were isolated from soil samples and determined the potency of microbes in producing cellulase and xylanase which were indicated by clear zones formation around the cultures and found maximum enzyme production at 30°C and pH of 6.0 in *Trichoderma atroviride* on 5th day of incubation. Mansfield et al. (1999) reported the *Trichoderma* spp. and *Aspergillus* spp. have most widely been used for production of cellulase and xylanase enzymes. Gautam et al. (2010) observed that the *Trichoderma* sp. is well known among the cellulolytic fungi for their potential to degrade organic municipal solid waste. Doolotkeldieva and Bobusheva (2011) have been reported the soft-rot fungi, *Trichoderma viride* and *Trichoderma reesei* are the most extensively studied cellulolytic fungi.

**IV Conclusion**

Thermophilic microbes are preparing high quality of compost with useful micro and macro elements in short duration. This observation would help in improvement of plant productivity and promotes the need of introducing methods of farming for better utility of organic amendments. Hence the different types of enzymes production by dominant thermophiles fungi involved in composts was detected and observed very promising role by these fungi for decomposition of composts and found passive and negative enzyme activity. Hence, the fortification of organic wastes and their composts as a source of organic nutrients are imperative for sustainable agriculture.

**References**

1. **Allen PJ, Emerson R, Guayule Rubber.1949.** Microbiological improvement by shrub retting. *Ind Eng Chem.*:41:346–365. https://www.osti.gov/servlets/purl/1670867.
2. Apinis AE. 1963. Thermophilous fungi of coastal grasslands in soil organisms, proceedings of the colloquium on soil fauna, soil micro flora and their relationships. Doeksen J and Van der Drift J(ed). North Holland publ, Amsterdam, pp.127-938.

3. Aro N., Pakula T, Penttila M. 2005. Transcriptional regulation of plant cell wall degradation by filamentous fungi. FEMS Microbiol Rev., 29:719-39. doi:10.1016/j.femsre.2004.11.006

4. Asghar, HN, Ishaq M, Zahir ZA, Khalid M, Arshad M. 2006. Response of radish to integrated use of nitrogen fertilizer and recycled organic waste. Pak J Bot, 38: 691-700. t: https://www.researchgate.net/publication/228618399

5. Atkinson CF, DD Jones, Gauthier JJ. 1997. Microbial activities during composting of pulp and papermill primary solids, World Journal of Microbiology and Biotechnology 13(5): 519-525. doi: 10.1023/a:1018557123868

6. Bairagi S. 2016. Isolation, Screening and Selection of Fungal Strains for Potential Cellulase and Xylanase Production, International Journal of Pharmaceutical Science Invention. 5 (3):1-6. http://ijpsi.org/Papers/Vol5(3)/A05030106.pdf

7. Barnett HL, Hunter BB. 1972. Illustrated Genera of Imperfect Fungi. Burgess Publishing Company, Minneapolis, Minnesota, p.20 &241.

8. Balasundaran M. 2008. Development Of Microbial Inoculants For Aerobic Composting. KFRI Research Report No. 324 (Final Report of the Project KFRI 390/03). Biotechnology Discipline Division of Sustainable Natural and Plantation Forest Management Kerala Forest Research Institute (KFRI), Trichur, Kerala, pp.-i-ii & 1-32.

9. Bending GD, and Read DJ. 1997. Lignin and soluble phenolic degradation by ectomycorrhizal and ericoid mycorrhizal fungi. Mycol. Res. 107: 1348-1354.

10. Betts WB, Dart RK. 1988. The degradation of lignin related compounds by Aspergillus flavus. Journal of General Microbiology, 134, 2413-2420.

11. Bhalla A, Bansal N, Kumar S, Bischoff KM, Sani RK. 2013. Improved lignocellulose conversion to biofuels with thermophilic bacteria and thermostable enzymes: a review. Bioresour Technol, 128:751-59. doi: 10.1016/j.biortech.2012.10.145

12. Bhale UN, Rajkonda JN. 2012. Enzymatic activity of Trichoderma species. Novus Natural Science Research, 1(4):1-8. https://www.researchgate.net/publication/312493288_Enzymatic_activity_of_Trichoderma_species

13. Cihanog N, Sarikaya E. 2004. Investigation of lipase production by a new isolate of Aspergillus sp. World Journal of Microbiology and Biotechnology. 20: 193-197. doi: 10.1023/B:WIBI.0000021781.61031.3a

14. De Azeredo LAI, Gomes PM, Sant’Anna Jr. GL, Castilho LR, Freire DMG. 2007. Production and regulation of lipase activity from Penicillium restrictum in submerged and solid-state fermentations. Current Microbiology. 54: 361-365. doi: 10.1007/s00284-006-0425-7.
15. Dingle J, Reid WW, Solomans GL. 1953. The enzyme degradation of pectin and other polysaccharides II, Application of the ‘cup plate assay’ to the estimation of enzymes. J. Sci. Food Agric., 4:149-155. https://doi.org/10.1002/jsfa.2740040305

16. Dominguez A, Pastrana L, Longo MA, Rua ML, Sanroman MA. 2005. Lipolytic enzyme production by Thermus thermophilus HB27 in a stirred tank bioreactor. Biochemical Engineering Journal. 26: 95-99. https://doi.org/10.1016/j.bej.2005.04.006

17. Dix NJ, Webster J.1995. Fungal Ecology. Chapman & Hall, London, pp. 549. https://dokumen.tips/documents/fungal-ecology-n-j-dix-and-j-webster-chapman-hall-london-1995-pp.html

18. Doolotkeldieva TD, Bobusheva ST. 2011.Screening of wild-type fungal isolates for cellulolytic activity. Microbiology Insights. 4: 1-10. https://doi.org/10.4137/MBI.S6418

19. Gautam SP, Bundela PS, Pandey AK, Awasthi MK, Sarsaiya S. 2010.Screening of cellulolytic fungi for management of municipal solid waste, J App Sci Environ Sanit. (4):391-395. http://ijpsi.org/Papers/Vol5(3)/A05030106.pdf

20. Haki GD, Rakshit SK. 2003. Developments in industrially important thermostable enzymes: a review. Bioresour Technol, 89: 17-34. doi: 10.1016/s0960-8524(03)00033-6.

21. Hankins L, Anagnostakis SL. 1975. The use of solid media for the detection of enzyme production by fungi. Mycologia. 67:597-607. https://doi.org/10.2307/3758395

22. Harper SHT, Lynch JM. 1985. Colonization and decomposition of straw by fungi. Transactions of British Mycological Society, 85:655-661. https://doi.org/10.1016/S0007-1536(85)80260-6

23. Johri BN, Jain S, Chauhan S. 1985. Enzymes from thermophilic fungi. Proteases and lipases. Proc. Indian Acad. Sci. (Plant Sci). 94:175-196. https://www.ias.ac.in/public/Volumes/plnt/094/02-03/0175-0196.pdf

24. Johri BN, Satyanarayana T, Olsen J. 1999. Thermophilic moulds in Biotechnology. Kluwer Academic Publishers, USA.

25. Joshi AA, Kanekar PP, Kelkar AS, Shouche YS, Vani AA, Borgave SB, Sarnaik SS. 2008. Cultivable bacterial diversity of alkaline Lonar Lake, India. Microbial Ecology. 55:163-172.

26. Kumar RR, Sreelatha B, Girisham S, Reddy SM. 2010. Incidence of thermophilic fungi from different substrates in Andhra pradesh (India). International Journal of Pharma and Bio sciences, 1(3): 1-6.

27. Lee H, Young Min Lee, Yeongseon Jang, Sangjoon Lee, Hwanhi Lee, Byoung Jun Ahn, Gyu-Hyeok Kim and Jae-Jin Kim. 2014. Isolation and Analysis of the Enzymatic Properties of Thermophilic Fungi from Compost. Mycobiology.42(2):181-184. doi: 10.5941/MYCO.2014.42.2.181

28. Maheshwari Ramesh, Girish Bharadwaj.Bhat MK. 2000.Thermophilic Fungi: Their Physiology and Enzymes. Microbiol Mol Biol Rev. 64(3):461–488. doi: 10.1128/mmbr.64.3.461-488.2000
29. Mansfield SD, Mooney C, Saddler JN. 1999. Substrate and enzyme characteristics that limit cellulose hydrolysis, Biotechnological Progress. 15: 804–816. doi: 10.1021/bp9900864.

30. Msaraha Marwan, Izyanti Ibrahimb, Wan Syaidatul Aqma. 2018. Enzyme activity screening of thermophilic bacteria isolated from Dusun Tua Hot Spring, Malaysia AIP Conference Proceedings 1940,020070.

31. Naik PS. 2017. Studies on microbial consortia for production and Enrichment of bio-compost from grapevine residues. Master Of Science (Agriculture) Thesis submitted to the Department of Agricultural Microbiology college of Agriculture, Dharwad University of Agricultural Sciences, Dharwad- 580 005.

32. Nakamura H, Kobayashi J, Nakamura Y, Ohizumi Y, Kondo T, Hirata Y. 1993. Theonellamine B, a novel peptidal Na,K-ATPase inhibitor from an Okinawan marine sponge of the genus Theonella. Tetrahedron Letters. 27:4319-4322.

33. Papdiwal PB. 1982. Pectolytic enzymes. In: Methods in experimental plant pathology (eds) Mukadam, D. S. and Gangawane, L. V. Marathwada University Press Aurangabad. pp.14-18.

34. Rajasekaran AK, Maheshwari, R. 1990. Growth kinetics and intracellular protein breakdown in mesophilic and thermophilic fungi. Indian J Exp Biol. 28: 46–51. https://doi.org/10.1016/S0953-7562(09)80144-5

35. Rastogi G, Bhalla A, Adhikari A, Bischoff KM, Hughes SR, Christopher LP, Sani RK. 2010. Characterization of thermostable cellulases produced by Bacillus and Geobacillus strains. Bioresour Technol, 101: 8798-06. doi: 10.1016/j.biortech.2010.06.001.

36. Sahu S, Pramanik., K. 2015. Delignification of cotton gm waste and its optimization by using white rot fungus Pycnoporous cinnabarinus. J. Environ. Biol. 36: 661-667. doi: 10.19080/AIBM.2017.05.5556674

37. Salar RK, Aneja KR. 2007. Thermophilic Fungi: Taxonomy and Biogeography. Journal of Agricultural Technology. 3(1): 77-107. http://www.ijat-aatsea.com/pdf/jun_v3_07/8-ijat2007_04-r.pdf

38. Satyanarayana T, Johri BN, Klein J. 1992. Biotechnological potential of thermophilic fungi. In: D. K. Arora, R. P. Elander, and K. G. Mukherji (eds.), Handbook of Applied Mycology. Marcel Dekker Inc, New York. pp. 729-761. doi: 10.1007/978-94-015-9206-2_3

39. Sierra GA. 1957. A simple method for the detection of lypolytic activity of microorganisms and some observations on the influence of the contact between cells and fatty substracts. Antonine van Leeuwenhoek, 28:15-22. doi: 10.1007/BF02545855.

40. Singh BS, Saxena., SB. 1982. Extracellular amylolytic activity of Penicillia. J. Indian Bot. Soc. 61:216-220.

41. Singleton P, Sambury, D. 1998. Dictionary of Microorganisms. 4th edition John Willey and Sons Press, New York; p. 1017.
42. **Subramanian CV.1971.** Hypomycetes: an account of Indian species except cercosporae. Indian Council of Agricultural Research, New Delhi. p.463.

43. **Trojanowski J, Haider K, Sundman V. 1970.** Decomposition of 14C labeled lignin and phenols by a *Nocardia* sp. *Archives of Microbiology*, 114:149-153. https://doi.org/10.1007/BF00410776

44. **Viikari L, Alapuranen M, Puranen T, Vehmaanpera J, Siika-Aho, M. 2007.** Thermostable enzymes in lignocellulose hydrolysis. *Adv Biochem Eng Biotechnol*, 108: 121-45. DOI: 10.1007/10_2007_065

45. **Waksman SA, Umbreit WW, Cordon TC. 1939.** Thermophilic actinomycetes and fungi in soils and in composts. *Soil Science*, 47: 37-62. https://doi.org/10.1097/00010694-193902000-00001

46. **Zambare VP, Bhalla A, Muthukumarappan K, Sani RK, Christopher LP. 2011.** Bioprocessing of agricultural residues to ethanol utilizing a cellulolytic extremophile. *Extremophiles*. 15: 611-18. doi: 10.1007/s00792-011-0391-2.

47. **Yeoman CJ, Han Y, Dodd D, Schroeder CM, Mackie RI, Cann IK. 2010.** Thermostable enzymes as biocatalysts in the biofuel industry. *Adv Appl Microbiol*. 70: 1-5. doi: 10.1016/S0065-2164(10)70001-0.