



American Journal of
Food Technology

ISSN 1557-4571



Academic
Journals Inc.

www.academicjournals.com

Optimization of Xylanase Production from *Fusarium solani* F7

¹V.K. Gupta, ¹R. Gaur, ²N. Gautam, ²P. Kumar, ¹I.J. Yadav and ¹N.S. Darmwal
¹Dr. R.M.L. Awadh University, Faizabad- 224001, UP, India
²Department of Biotechnology, BBA University, Lucknow, UP, India

Abstract: The purpose of this study was to characterization of xylanase producing *Fusarium solani* isolate and optimization of cultural conditions for xylanase enzyme production. Screening of *Fusarium solani* isolate was based on the diameter of the clear zone formation in oat spelt xylan agar plates, *Fusarium solani* isolate F7 was selected and optimized for xylanase enzyme production using cheaper substrate like wheat straw, rice straw, rice bran and wood husk. Maximum enzyme activity was observed in wheat straw (78.32 U mL⁻¹). Optimum pH and temperature for xylanase activity were found to be 5.5 and 30°C at 3% substrate concentration. In purification step, 75% ammonium sulphate saturation was found to be suitable giving maximum xylanase activity. Purified xylanase yielded single band with a molecular weight of 89 kDa. The use of wheat straw as a major carbon source is particularly valuable because oat spelt xylan is very expensive, The *Fusarium solani* F7 isolate proved to be a promising microorganism for xylanase production.

Key words: Xylanase production, *Fusarium*, substrate, carbon source, nitrogen source

INTRODUCTION

Xylans are the major hemicelluloses in wood from angiosperms, where they account for 15-30% of the total dry weight. In the gymnosperms however, xylans contribute only 7-12% of the total dry weight. The structure of xylans is complex and their complete biodegradation requires the concerted action of xylanases enzymes. Xylans are heterogeneous polysaccharides with a backbone consisting of beta-1, 4 linked D-xylosyl residues. Endo-beta-1, 4 xylanases are the main enzymes responsible for cleavage of the linkages within the xylan backbone (Amani *et al.*, 2007). To date xylanase has gained increasing attention because of its various biotechnological applications and have great potential for industrial applications mainly for the bioconversion of lignocelluloses to sugar, ethanol and other useful substances (Viikari *et al.*, 1994). They are extensively used in pre-treatment of forage crops and other lignocellulosic biomass, added to swine and poultry cereal-based diets to improve nutrient utilization, flour modification for bakery products and saccharification of agricultural, industrial and municipal wastes (Sá-Pereira *et al.*, 2002). Moreover, it is reported that xylanases have been widely used for clarifying fruit juices and wine (Hang and Woodams, 1997), food processing industries and in the production of several valuable products like xylitol and ethanol (Salles *et al.*, 2005) and improving the nutritional properties of agricultural silage and grain feed (Kuhad and Sing, 1993). To reach commercial feasibility, enzyme production must be increased by introducing a more potent strain and by optimizing culture conditions. Xylanases are produced by numerous microorganisms among which the fungi are the most potent producers (Pham *et al.*, 1997). Haltrich *et al.* (1996) gave an overview of fungal xylanases and showed that the enzyme can be produced by a number of microorganisms including bacteria, yeasts and filamentous fungi such as *Trichoderma*, *Aspergillus*, *Penicillium*, *Fusarium*, *Chaetomium*, *Humicola*, *Talaromyces* and many others. *Fusarium oxysporum* and other

Fusarium sp. has been shown to be a promising organism for enhanced production of xylanases (Chirstakopoulos *et al.*, 1999). Extracellular enzymes are considered important from the industrial viewpoint as they ease the extraction procedure. The use of purified xylan as a substrate to induce xylanase synthesis increases the cost of enzyme production. Therefore, for commercial applications, there have been attempts to develop a bioprocess to produce xylanase in high quantities from simple and inexpensive substrates. Wheat straw, wheat bran and corncob has been shown to be an efficient substrate in the production of xylanase and the enzyme production is related to the type and concentrations of nutrients and growth conditions (Alam *et al.*, 1994; Hoq and Deckwer, 1995; Haltrich *et al.*, 1996; Gawande and Kamat, 1999; Kang *et al.*, 2004; Sonia *et al.*, 2005). Filamentous fungi are interesting procedures of this enzyme from an industrial point of view due to extracellular release of xylanases, higher yield compared to bacteria and yeast and production of several auxiliary enzymes that are necessary for debranching of the substituted xylanase (Haltrich *et al.*, 1996).

Thus, since there is less reports on efficient xylanase production by the fungus *Fusarium solani*, therefore the objective of this study to consolidate information on optimization process for xylanase production.

MATERIALS AND METHODS

Isolation of *Fusarium solani* Isolate

The soil sample for the isolation of *Fusarium solani* were collected from root zone of different nearby areas of Faizabad region, U.P., India (Lat. 26°47' N; Long. 82°12' E). The *Fusarium solani* culture were isolated from soil samples by serial dilution method of Clark *et al.* (1988), 1 g soil sample was dissolved in 100 mL sterilized distilled water. The soil suspension was diluted up to 10⁺³ to 10⁻³ and 0.5 mL of diluted suspension was used. The study was carried out at Microbiology Laboratory, Dr. R.M.L. Awadh University, Faizabad-224001, UP, India during 2007-2008.

Identification of *Fusarium solani* Isolate

Identification was based on cell and colony morphology characteristics as per method described by Booth (1971). The young colonies of *Fusarium solani* were aseptically picked up and transferred to PDA slants and incubated at 28±2°C for 4-5 days for maximum growth.

Screening for Xylanolytic Activities

The fungal isolates formed were subcultured to purity and examined for xylanolytic activities. Screening for xylanolytic activities was performed on Malt Extract Agar (MEA) containing 0.1% (w/v) of xylan from oat spelt. Positive xylanolytic isolates were detected based on the clear zones of hydrolysis on the xylan. The potential isolates were subcultured and maintained on MEA slants. The slants were stored at 4°C prior to use.

Xylanase Production Medium

Erlenmeyer flasks (250 mL) containing 10 g of wheat straw were added with the Mandels and Sternburg's basal medium (Mandels and Sternburg, 1976) just to wet the wheat straw. The Mandel's medium was prepared with the following composition (g L⁻¹) 10.0 g; urea, 0.3; peptone, 0.75; yeast extract, 0.25; (NH₄)₂SO₄, 1.4; KH₂PO₄, 2.0; CaCl₂, 0.3; MgSO₄.7H₂O, 0.3 and trace elements (mg L⁻¹): FeSO₄.7H₂O, 5; MnSO₄.4H₂O, 1.6; ZnSO₄.7H₂O, 1.4 and CoCl₂.6H₂O, 20.0 and Tween 80 0.1% (v/v) (pH 5.0). The flasks were inoculated with 2 mL of spore suspension prepared from a week old PDA slants of the culture grown at 30°C. Sterile distilled water containing 0.1% (v/v) Tween 80 was used to prepare fungal spore suspension. Inoculated flasks were incubated at 30°C under static conditions for 10 days.

Inoculum and Fermentation

A inoculum size of conidia (Each mL of cells suspension contained 2.0×10^6 cells) was transferred from a stock culture in 250 mL flask containing 50 mL of growth medium. The flasks were incubated for 72 h at $28 \pm 2^\circ\text{C}$ on a rotatory shaker at 150 rpm.

Preparation of Enzyme

The enzyme from each flask was extracted using 50 mL of 0.05M citrate buffer (pH 5.3) and filtered through a wet muslin cloth by squeezing. The extract was centrifuged at 5000 rpm for 20 min. The clear supernatant was partially purified by ammonium sulphate fractionation at 4°C to achieve 75% saturation and dialysed using the same buffer for 24 h with three intermittent changes.

Xylanase Assay

The supernatant was used as a source for enzyme sample. Xylanase activity was measured with the optimized method described by Bailey *et al.* (1992), using 1% oat spelt xylan, (Himedia, Mumbai, India) as the substrate. The solution of xylan and the enzyme at appropriate dilution were incubated at 55°C for 7 min and the reducing sugars were determined by the dinitrosalicylic acid method described by Miller (1959), with xylose as standard. The released xylose was measured spectrophotometrically at 540 nm. One unit of xylanase was defined as the amount of enzyme (protein) in milligram required for hydrolysis of starch to produce a millimolar of reducing sugar in 1 h under above assay conditions. The specific activity was defined as number of units per gram protein. All experiments were repeated thrice.

SDS-PAGE of Xylanase Enzyme

The cell free supernatant of crude enzyme was assayed on sodium dodecyl sulphate-poly acrylamide gel electrophoresis for protein profiling to confirm the molecular weight of xylanase enzyme.

Optimization of Process Parameters

The optimization of composition of medium and cultural conditions was carried out based on stepwise modification of the governing parameters for xylanase production. The effect of various substrates, consisting of rice straw, wheat straw, rice bran and wood husk was examined by adding 10 g of each substrate in a 250 mL Erlenmeyer flask with 10 mL of sterile distilled water, which was added to moisture the substrates. Cultivation was carried out at ambient temperature ($28 \pm 2^\circ\text{C}$) for 7 days. Changing the pH 3 to 8 in the production medium the effect of pH was observed. The effect of cultivation temperature on the enzyme production was examined at different temperatures starting from 25 to 50°C with 5°C intervals. The effects of incubation period were evaluated by 24 h interval by checking the enzyme activity. The optimization was carried out by adding standardized concentration wheat straw using supplemented carbon sources like glucose, sucrose, galactose, starch, dextrin, xylose, sorbitol, carboxymethyl cellulose, fructose and nitrogen sources like were peptone, urea, yeast extract, sodium nitrate, ammonium sulphate, ammonium nitrate and beef extract, were also tried.

RESULTS AND DISCUSSION

The application of xylanases are widely spread in paper and pulp industries as well as in food and pharmaceutical industries. In present investigation, purification of xylanase was carried out. The molecular weight of the single protein band was calculated to be about 89 kDa (Fig. 1). Microbial xylanases are single subunit proteins with molecular masses within a the range of 8-145 kDa

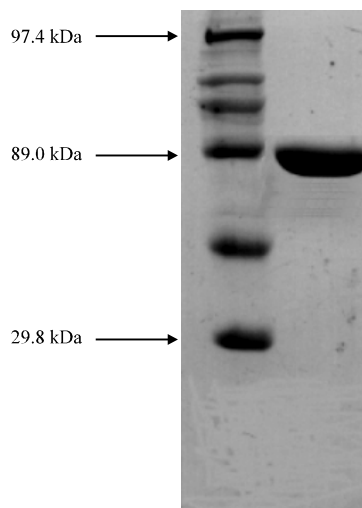


Fig. 1: Purification of xylanase from *Fusarium solani* F7

(Kulkarni *et al.*, 1999). In a earlier study, a purified xylanase enzyme preparation also showed a single protein band on sodium dodecyl sulphate-poly acrylamide gel electrophoresis and the molecular weight of this enzyme was found to be 24 kDa (Sardar *et al.*, 2000).

Selection and Identification of Isolate *Fusarium solani*

Based on the screening programme, a total of 16 isolates were capable of exhibiting xylanolytic activities on MEA-xylan agar with the diameter of the clear zones ranging from 34 to 48 mm. However, 5 isolates consisting of F2, F5, F7, F11, F13 and F15 were selected for further confirmation using the MEA-xylan agar plates. The isolates F7 demonstrated reproducible zones of hydrolysis of 48 mm diameter and this isolate was selected as the potential producer of xylanase. Isolate F7 was identified based on the structural morphologies as observed under the light and scanning electron microscopes. It was observed that the isolate possessed distinct macro and micro conidia formation. Macro-conidia were of 3-5 celled slightly curved at the pointed ends, typically canoe shaped whereas micro-conidia 1-celled, ovoid. Based on these characteristics, isolate *Fusarium solani* isolates were identified to be *Fusarium solani* (Booth, 1971) and isolate F7 was used for further investigation for optimization of xylanase producing property.

Effect of Incubation Period

The xylanase activity was determined after every 24 h of incubation in order to determine the optimum incubation period for maximum production of xylanase. The enzyme production however, started after 24 h of inoculation and showed maximum production (56.31 U mL^{-1}) on 6th day of incubation period at 30°C (Fig. 2). In some fungi, high xylanase production has been shown to be linked strictly to cellulose production due to time course or incubation period (Haltrich *et al.*, 1996; Chirstakopoulos *et al.*, 1999; Kang *et al.*, 2004).

Effect of Temperature

Temperature is one of the important parameters that determine the success of optimization system. Therefore, the effect of temperature on xylanase production by *Fusarium solani* F7 was examined at various temperature ranges 20 to 50°C for 48 h and the results obtained are shown

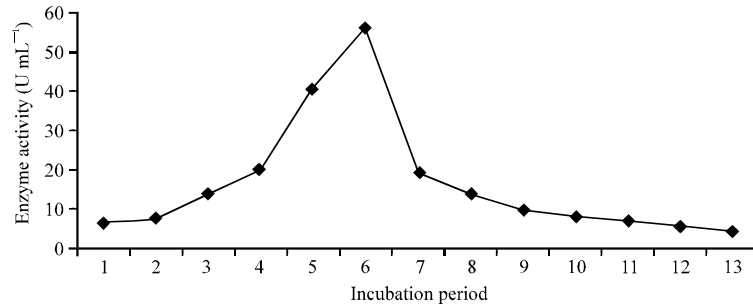


Fig. 2: Effect of incubation period on xylanase production

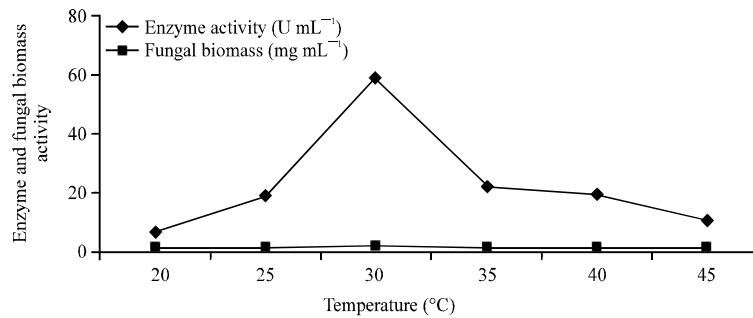


Fig. 3: Effect of temperature on production of xylanase and fungal biomass

in Fig. 3. The production of xylanase was maximum at the temperature near to ambient temperature (30°C) with an activity of 58.8 U mL⁻¹ and the growth about 2.2 mg mL⁻¹. A lower activity in the range of 6.42-22.11 U mL⁻¹ and growth of about 1.10-1.42 mg mL⁻¹ substrate were obtained with cultivation temperatures lower or above the ambient temperature. At 20°C, the activity of xylanase was 6.42 U mL⁻¹. The results obtained indicated that the enzyme production corresponded closely to the growth of the fungus. The optimum temperature for xylanase production is similar to the optimum temperature for the growth of the fungus. This observation was in agreement with those reported by Chirstakopoulos *et al.* (1999) and Biswas *et al.* (1988, 1990), who showed that the highest xylanase activities were obtained at temperatures that were optimum for the growth of the fungi. *Fusarium solani* F7, the ambient temperature which was the optimum temperature for xylanase production was similar to the temperature of the natural habitat of the fungus where it was initially isolated.

Effect of pH

The initial pH of medium was adjusted to variable pH range by adding the 0.1 N HCl. Enzyme purified was tested in the pH range (pH 3 to 8). The production of xylanase was found to be the best at pH 5.5 Below and above this pH production of xylanase was significantly lower (Fig. 4). The results showed that xylanase production by *Fusarium solani* F7 was much dependent on pH and the optimum initial pH was between pH = 5.0 and 5.5 (Fig. 4). However, when the pH was increased or decreased to values other than 5.0 and 5.5, the production of xylanase gradually decreased. This might be due to the fact that alkaline pH has inhibitory effect on the growth of *Fusarium solani* F7 (an acidophilic fungi) and enzyme production. xylanases from different organisms show an optimum pH within a range of 4.0-7.0. However, certain xylanases from *Aspergillus kawachii*, *Penicillium herque* and some

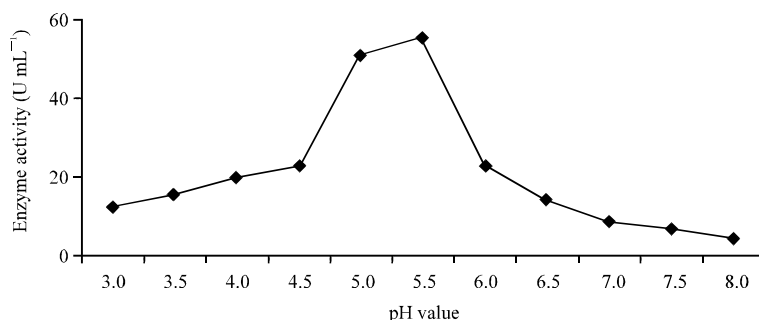


Fig. 4: Effect of pH on enzyme activity

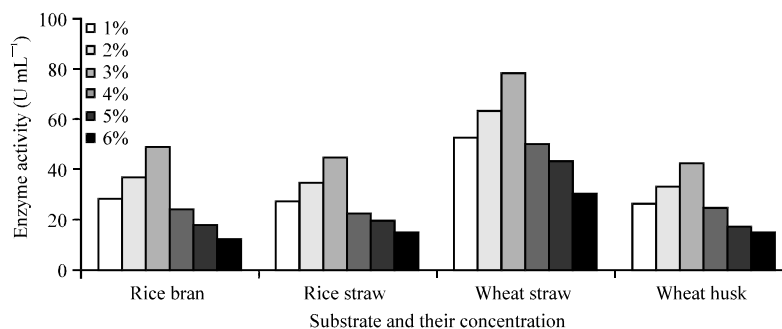


Fig. 5: Production of xylanase influenced by substrates and their concentration

other fungi including *Fusarium oxysporum* exhibit an optimum pH more on the acidic side (pH 2.0-6.0) (Funaguma *et al.*, 1991; Ito *et al.*, 1992; Kulkarni *et al.*, 1999). Endoxylanase 1 and 2 from *Aspergillus awamori* show an optimum pH at 5.5-6.0 and 5.0, respectively (Kormelink *et al.*, 1992).

Effect of Substrate and Their Concentration

Standard xylanase activity (52.81 U mL⁻¹) was obtained in the medium containing 1% wheat straw after 144 h of incubation in comparison with the, Rice Straw, Rice bran and wood husk (Fig. 5). In order to determine the best amount of wheat straw for xylanase production, different concentrations (1-6% by mass per volume) were tested.

The results showed that the highest yield of xylanase was 78.32 U mL⁻¹ with 3% wheat straw (Fig. 5). Increasing the concentration for more than 3% resulted in a significant decrease in xylanase activity. This might be attributed to the fact that high concentration of substrate led to the increase in medium viscosity, which influenced the mixture medium components and oxygen transfer. Similar results were obtained by other researchers by using high concentrations of lignocellulosic materials as substrates for enzyme production (Cao *et al.*, 2008). Wheat straw and wheat bran has been known for being ideally suitable for xylanase production in *T. aurantiacus* and *Penicillium citrinum* cultures (Kalogeris *et al.*, 1998; Nair *et al.*, 2008).

Effect of Supplemented Carbon Source

The production of primary metabolites by microorganisms is highly influenced by their growth, which is determined by the availability of the nutrients in the substrates. Therefore, it is expected that the improvement of the nutritional value of wheat straw by the supplementation of carbon will also

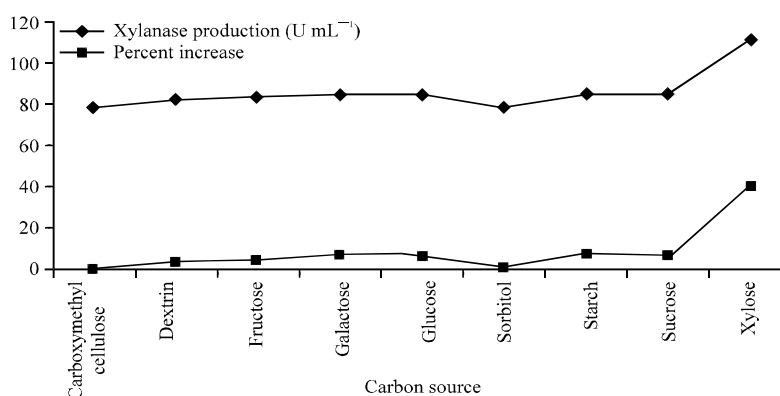


Fig. 6: Production of xylanase influenced by t carbon sources with 3% wheat straw as substrate

improve the growth of *Fusarium solani* F7 and subsequently the enzyme production. Flask containing production media supplemented with carbon sources (glucose, sucrose, starch, carboxymethyl cellulose, fructose, sorbitol, xylose, galactose and dextrin) were tested for the influence of carbon sources at 1% concentration. Figure 6 shows the supplementation of sugars, which may act either as carbon sources or inducers. Starch, Sucrose and dextrin, fructose and sorbitol and galactose were moderate source carbon sources for xylanase production. Carboxymethyl cellulose was poor producer of xylanase (Fig. 6). As shown in the Fig. 6, the addition of xylose resulted in an increment of 42% in xylanase production from other supplemented carbon sources. These findings confirmed that the size of carbon source was an important factor in xylanase production (Kalogeris *et al.*, 1998).

Xylose has been described as an effective inducer and carbon source for xylanase production in several microorganisms for xylanase production including *A. pullulans* (Priem *et al.*, 1991), *Fusarium oxysporum* (Chirstakopoulos *et al.*, 1999) and *T. lanuginosus* (Purkarthofer *et al.*, 1993). Xylan is costly for large-scale production of xylanases, lignocellulosic materials can be used as cost-effective substrates for xylanase production (Haltrich *et al.*, 1996; Beg *et al.*, 2000). Various lignocellulosic materials and microbial cultures have been used successfully in solid-state fermentation for xylanase production (Topakas *et al.*, 2003; Sonia *et al.*, 2005). The significant difference in xylanase titers, when wheat straw was used as the carbon source may be attributed to its hemicellulose nature and favorable degradability, the presence of some nutrients in the carbon source (Sonia *et al.*, 2005).

Effect of Supplemented Nitrogen Source

Effect of different nitrogen sources viz. peptone, urea, yeast extract, sodium nitrate, ammonium sulphate, ammonium nitrate and beef extract on the production of xylanase was studied, it was observed that ammonium sulphate, yeast extract and beef extract caused poor enzyme production. Peptone and sodium nitrate supported maximum production of enzyme whereas ammonium nitrate and urea produced considerable amount of xylanase. The optimum concentration of peptone was 0.3% (Fig. 7). The results obtained also did not exhibit any significant difference between the organic or inorganic nitrogen sources on the production of xylanase by *Fusarium solani* F7. These results are in agreement with those reported in the literature where fungi were found to produce higher xylanase activities on organic nitrogen sources (Purkarthofer *et al.*, 1993; Lemos *et al.*, 2001).

The mineral salt solution, distilled water, organic nitrogen source solution and mineral salt solution+ organic nitrogen source solution used as MA supported xylanase production and extracellular protein synthesis to varying degrees. The effects of several MAs have been reported on xylanases

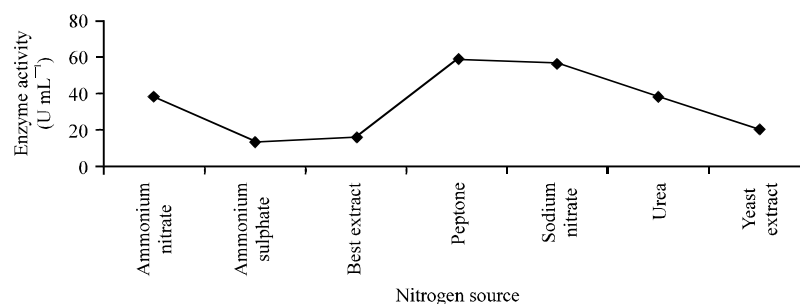


Fig. 7: Effect of different nitrogen sources at 0.3% concentrations

production in SSF (Grajek, 1986, 1987; Alam *et al.*, 1994; Gutierrez-Correa and Tengerdy, 1998; Chirstakopoulos *et al.*, 1999).

CONCLUSION

The results obtained from the present study indicates that significant improvement of xylanase production by *Fusarium solani* F7 isolate could be obtained by selective use of nutrients and growth conditions. Since oat spelt xylan is an expensive substrate for commercial scale xylanase production, the possibility of using wheat straw for xylanase production was investigated. Wheat straw (3% by mass per volume) could be used as a less expensive substrate for efficient xylanase production (78.32 U mL⁻¹). This observation is interesting due to the low cost of these carbon sources.

ACKNOWLEDGMENTS

We are thankful to Head, Department of Microbiology, Dr. R.M.L. Awadh University for research support to complete this study. Dr. L.P. Yadava, Central Institute for Sub-Tropical Horticulture, Lucknow, UP, India for providing the research guidance.

REFERENCES

- Alam, M., I. Gomes, G. Mohiuddin and M.M. Hoq, 1994. Production and characterization of thermostable xylanases by *Thermomyces lanuginosus* and *Thermoascus aurantiacus* grown on lignocelluloses. *Enzyme Microb. Tech.*, 16: 298-302.
- Amani, M.D., E.I. Ahwany and A.S. Youssef, 2007. Xylanase production by *Bacillus pumilus*: Optimization by statistical and immobilization methods. *Res. J. Agric. Biol. Sci.*, 3: 727-732.
- Bailey, M.J., P. Biely and K. Poutanen, 1992. Interlaboratory testing of methods for assay of xylanase activity. *J. Biotechnol.*, 23: 257-270.
- Beg, Q.K., B. Bhushan, M. Kapoor and G.S. Hoondal, 2000. Enhanced production of a thermostable xylanase from *Streptomyces* sp. QG-11-3 and its application in biobleaching of eucalyptus kraft pulp. *Enzyme Microb. Technol.*, 27: 459-466.
- Biswas, S.R., A.K. Mishra and G. Nanda, 1988. Xylanase and xylosidase production by *Aspergillus ochraceus* during growth on lignocelluloses. *Biotechnol. Bioeng.*, 31: 613-616.
- Biswas, S.R., S.C. Jana, A.K. Mishra and G. Nanda, 1990. Production, purification and characterization of xylanase from a hyperxylanolytic mutant of *Aspergillus ochraceus*. *Biotechnol. Bioeng.*, 35: 244-251.

- Booth, C., 1971. The Genus *Fusarium*. 1st Edn., Commonwealth Mycological Institute, Kew, England, ISBN: 85198 046 5.
- Cao, Y., M. De-Jing, J. Lu and J. Long, 2008. Statistical optimization of xylanase production by *Aspergillus niger* AN-13 under submerged fermentation using response surface methodology. *Afr. J. Biotechnol.*, 7: 631-638.
- Chirstakopoulos, P., D. Mamma, W. Nerinckxw, D. Kekos and B. Macris *et al.*, 1999. Production and partial characterization of xylanase from *Fusarium oxysporum*. *Bioresour. Technol.*, 58: 115-119.
- Clark, H.E., E.F. Geldrich, P.W. Kabler and C.B. Huff, 1988. *Applied Microbiology*. 1st Edn. International Book Company, New York, pp: 53.
- Funaguma, T., S. Naito, M. Morita, O.M. Kumara and M. Sugiura *et al.*, 1991. Purification and some properties of xylanase from *Penicillium herquei* Banier and Sartory. *Agric. Biol. Chem.*, 55: 1163-1165.
- Gawande, P.V. and M.Y. Kamat, 1999. Production of *Aspergillus xylanase* by lignocellulosic waste fermentation and its application. *J. Applied Microbiol.*, 87: 511-519.
- Grajek, W., 1986. Temperature and pH optimum of enzyme activities produced by cellulolytic thermophilic fungi in batch and solid-state cultures. *Biotechnol. Lett.*, 8: 587-590.
- Grajek, W., 1987. Production of D-xylanases by thermophilic fungi using different methods of culture. *Biotechnol. Lett.*, 9: 353-356.
- Gutierrez-Correa, M. and R.P. Tengerdy, 1998. Xylanase production by fungal mixed culture solid substrate fermentation on sugar cane bagasse. *Biotechnol. Lett.*, 209: 45-47.
- Haltrich, D., B. Nidetzky, K.D. Kulbe, W. Steiner and S. Zupancic, 1996. Production of fungal xylanases. *Bioresour. Technol.*, 58: 137-161.
- Hang, Y.D. and E.E. Woodams, 1997. Xylanolytic activity of commercial juice processing enzyme preparations. *Lett. Applied Microbiol.*, 24: 389-392.
- Hoq, M.M. and W.D. Deckwer, 1995. Cellulase-free xylanase by thermophilic fungi: A comparison of xylanase production by two *Thermomyces lanuginosus* strains. *Applied Microbiol. Biotechnol.*, 43: 604-609.
- Ito, K., J. Ogassawara, T. Sugimoto and T. Ishikawa, 1992. Purification and properties of acid stable xylanases from *Aspergillus kawachii*. *Biosci. Biotechnol. Biochem.*, 56: 547-550.
- Kalogeris, E., P. Christakopoulos, D. Kekos and B.J. Macris, 1998. Studies on the solid-state production of thermostable endoxylanases from *Thermoascus aurantiacus*: Characterization of two isozymes. *J. Biotechnol.*, 60: 155-163.
- Kang, S.W., Y.S. Park, J.S. Lee, S.I. Hong and S.W. Kim, 2004. Production of cellulase and hemicellulases by *Aspergillus niger* KK2 from lignocellulosic biomass. *Bioresour. Technol.*, 91: 153-156.
- Kormelink F.J.M., H. Gruppen, T.M. Wood and G. Beldman, 1992. Mode of Action of Xylan Degrading Enzymes From *Aspergillus awamori*. In: *Xylans and Xylanases*, Visser, J., G. Beldman, M.A. Kustersvan- Someren and A.G.J. Varagen, (Eds.). Elsevier, Amsterdam, London, New York, pp: 141-147.
- Kuhad, R.C. and A. Singh, 1993. Lignocellulose biotechnology: Current and future prospects. *Crit. Rev. Biotechnol.*, 13: 151-172.
- Kulkarni, N., A. Shendye and M. Rao, 1999. Molecular and biotechnological aspects of xylanases. *FEMS. Microbiol. Rev.*, 23: 411-456.
- Lemos, J.L.S., M.C.A. Fontes and N.J. Pereira, 2001. Xylanase production by *Aspergillus awamori* in solid-state fermentation and influence of different nitrogen sources. *Applied Biochem. Biotechnol.*, 93: 681-689.
- Mandels, M. and D. Sternburg, 1976. Recent advances in cellular technology. *J. Ferment. Tech.*, 54: 267-286.

- Miller, G.L., 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chem.*, 31: 426-428.
- Nair, S.G., R. Sindhu and S. Shashidhar, 2008. Fungal xylanase production under solid state and submerged fermentation conditions. *Afr. J. Microbiol. Res.*, 2: 82-86.
- Pham, P.L., P. Taillandier, M. Delmas and P. Strehaiano, 1997. Optimization of a culture medium for xylanase production by *Bacillus* sp. using statistical experimental designs. *World J. Microbiol. Biotechnol.*, 14: 185-190.
- Purkharthofer, H., M. Sinner and W. Steiner, 1993. Cellulase-free xylanase from *Thermomyces lanuginosus*: Optimization of production in submerged and solid-state culture. *Enzyme Microb. Technol.*, 15: 677-682.
- Sá-Pereira, P., A. Mesquita, J.C. Duarte, M.R.A. Barros and M. Costa-Ferreira, 2002. Rapid production of thermostable cellulase-free xylanase by a strain of *Bacillus subtilis* and its properties. *Enzyme Microbiol. Technol.*, 30: 924-933.
- Salles, B.C., R.G. Medeiros, S.N. Bao, J.F.G. Silva and E.X.F. Filho, 2005. Effect of cellulase free xylanases from *Acrophialophora nainiana* and *Humicola grisea* var. *thermoidea* on eucalyptus kraft pulp. *Process Biochem.*, 40: 343-349.
- Sardar, M., I. Roy and M.N. Gupta, 2000. Simultaneous purification and immobilization of *Aspergillus niger* xylanase on the reversibly soluble polymer Eudragit™ L-100. *Enzyme Microbiol. Technol.*, 27: 672-679.
- Sonia, K.G., B.S. Chadha and H.S. Saini, 2005. Sorghum straw for xylanase hyper-production by *Thermomyces lanuginosus* (D₂W₃) under solid state fermentation. *Bioresour. Technol.*, 96: 1561-1569.
- Topakas, E., P. Katapodis, D. Kekos, B.J. Macris and P. Christakopoulos, 2003. Production and partial characterization of xylanase by *Sporotrichum thermophile* under solid-state fermentation. *World J. Microbiol. Biotechnol.*, 19: 195-198.
- Viikari, L., A. Kantelinen, J. Sundquist and M. Linko, 1994. Xylanases in bleaching: From an idea to industry. *FEMS Microbiol. Rev.*, 13: 335-350.