Production of Xylanase from *Aspergillus sydowii* Isolated from *Irvingia gabonensis* (African Mango) Fruit

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Abstract

This work reports xylanase production from *Aspergillus sydowii* isolated from spoiled *Irvingia gabonensis* fruit. Selection of the fungus was based on the production of maximum zone of clearing on agar containing 5% beech wood xylan. The influence of pure carbon substrates on enzyme production showed that the medium containing mannitol produced a maximum enzyme yield of 50.1 U/mg protein after incubation for 72 h. Enzyme productivity in this medium was much higher in a medium that contained only xylan (44.9 U/mg protein) as the sole carbon source. Nitrogen sources were tested for their abilities to stimulate enzyme production and the best enzyme production was observed in the medium containing peptone caused the production of specific enzyme activity of 41.2 U/mg protein after 72 h. The enzyme showed maximum activity (55 U/mg protein) at pH 4.0 and optimum stability at pH 3.0-5.0. Temperature activity profile of the enzyme showed best activity (68 U/mg protein) at 35^{o}C. Best temperature stability occurred at 30-40^{o}C. Dried agricultural residues were tested for their abilities to support xylanase production in media devoid of xylan. The overall best enzyme productivities of 97.8 and 92.6 U/mg protein were achieved after 72 h in the medium containing orange peel and mango peel respectively. These levels were much higher than that achieved when pure carbon sources or xylan alone were used for enzyme production.

Key words: Agricultural residues; carbon sources, nitrogen sources; pH activity; temperature activity

Introduction

Plant biomass is essentially lignocelluloses. Lignocelluloses are complex polymers of cellulose, hemicelluloses and lignin and represent the most abundant renewable organic matter on earth (Gupta *et al.*, 2000). Xylan is a major component of hemicelluloses and makes up about 30% of the dry weight of cell walls of monocotyledons (Wong *et al.*, 1998). Xylanases (E.C 3.2.1.8) are inducible enzymes which are responsible for hydrolysis of xylan.

Xylan is the most abundant non cellulosic material present in both hardwoods annual plants and accounts for 20 – 35% of the total dry weight in tropical plant biomass. Xylans (mainly β-1, 4-linked D-xylose) are major structural heteropolysaccharides in plants. They represent up to 30% of the dry weight of the cell walls of monocotyledons and constitute a minor component of dicotyledons (Wong *et al.*, 1998). They are second only to cellulose in abundance in plant material and are thus a major reserve of reduced carbon in the environment. Xylans belong to the group of complex structural polymers collectively referred to as hemicelluloses (Bastawde *et al.*, 1992). Recent interest in xylanase production and application is a welcome development, because xylanases are important in the bioconversion of hemicelluloses into their constituent sugars. Enzymatic hydrolysis of xylan in agricultural, industrial and municipal wastes may be used to obtain sugar supplements for producing speciality products such as the artificial sweetener, xylitol. For commercial applications, xylanases should ideally be produced quickly and in large quantities from simple and inexpensive substrates. Abundantly available agro-residues are an obvious source of substrate.

Microbial xylanases bring about most of the xylan degradation occurring in nature. This conversion of xylan to its constituent sugars; xylose, xylobiose and xylotriose is made possible by the action of various xylanolytic microorganisms such as *Trichoderma viride, Aspergillus* and *Bacillus* species. Microbial xylanases display several properties such as specificity of action, optimum pH and temperature. Microbial xylanases are produced by either solid state fermentation or submerged fermentation depending on the producer organisms. Factors such as suitable substrates and microorganisms favour production. Microbial xylanases can be applied in animal feed, juice, beer, silage and bread industries. With anticipated advances in biochemical engineering, improved bioprocess and molecular biology, it is expected that microbial xylanase producers will yield more xylanase for increased commercialization. Microbial xylanase deconstructs plant structural material by breaking down hemicelluloses, a major component of the plant cell wall. Plant cell walls are necessary to prevent dehydration and maintain physical integrity. They also act as a physical barrier to attack by plant pathogens. In nature, some plant consumers or pathogens use xylanase to digest or attack plants. Many microorganisms produce xylanase, but mammals do not. Some herbivorous insects and crustaceans also produce xylanase. Xylanase is the name given to a class of enzymes which degrade the linear polysaccharide β-1, 4-xylan into xylose, thus breaking down hemicelluloses, one of the major components of plant cell walls. Since last few years, there has been a growing interest for microbial xylanases as

they have important applications in the degradation of xylan. Substrate xylan, a biopolymer comprising of D-xylose monomers linked through 1, 4-glycosyl bond, is found abundantly in lignocellulosic biomass. Xylan molecules, are linked covalently with lignin phenolic residues, and are found to have interaction with polysaccharides, such as glucan and pectin. Xylans are linear homopolymers which have D-xylose monomers linked through β-1, 4-glycosyl bonds.

Microbes are attractive topic of interest for the production of xylanase due to their enzyme complexity and extreme habitat variability. Xylanases are produced by many different types of microorganisms such as *Trichoderma, Bacillus, Aspergillus, Penicillium, Schizophyllum, Aerobasidium,* and *Talaromyces* spp. (Seyis and Aksoz 2005). In similar research studies, *Aspergillus sydowii* has been reported as an effective producer of xylanase (Ghosh and Nanda 1994). Microbial xylanases are preferred for their vast industrial applicability and relatively lower cost of production. Medium components are responsible for the inducible production of xylanase, whereas enhanced production is obtained only in those media that contain pure xylan or xylan-rich substrates.

Potential application of xylanases in biotechnology is immense. It is produced and wasted annually in huge amounts and has several likely uses if suitably applied. Xylanase has been categorized as one of the industrially important enzymes, and the judicious used of xylanases in the industries could result in a cleaner reactions, higher yields, and lower consumption energy. Before xylanase can be used at industrial level, several criteria have to be fulfilled. Pilot scale industrial processes are carried out at high temperatures and by implication bacterial xylanases are preferred for use because of their broad ranges of pH and temperature stability (Kurakami *et al.*, 1999). In order to obtain maximal effect of xylanase, some of the reaction parameters such as enzyme dose, retention time, pH, and temperature should be optimized. The objective of this study was to evaluate the isolation, production and partial purification of xylanase obtained from *Aspergillus sydowii*. The effects of certain culture conditions on enzyme production were also studied. In this context, enzyme production on agricultural wastes in the absence of xylan in the production media was investigated in order to minimize raw material costs for enzyme production.

Materials and Methods

Isolation of fungi: Fruit from *Irvingia gabonensis* (African mango) undergoing spoilage was obtained near the tree and taken immediately into the laboratory for microbiological analysis. Spoiled sections (ca. 20 g) were removed with a sterile kitchen knife and homogenized in a mortar and pestle with 15 ml of sterile distilled water. The pH of the homogenate was 5.6. The homogenate was serially diluted with normal saline and plated out on Potato Dextrose agar (PDA) plates containing 0.1% chloramphenicol solution to inhibit bacterial contaminants. The plates were incubated for 48 h at room temperature (30±2^{o}C). Pure cultures of the isolates were obtained by streaking slant cultures on fresh PDA plates and were given arbitrary numbers.

Screening: Fungal isolate were each tested for xylanase activities by inoculating on plates containing agar agar and 2% beech wood xylan. Following three day incubation, the xylanolytic property of the colonies was assessed by flooding the plates with Congo red solution (1.0% w/v) for 15 min. and the plates were destained with 1 M NaCl solution (Shanthi and Roymon 2014). The colonies showing clear zone around them were selected for further studies. One fungal colony produced a maximum zone of clearance on the xylan agar was selected and identified as *Aspergillus sydowii* based on the taxonomic descriptions by Pitt and Hocking (1997).

Cultivation of the isolate and enzyme production: Spores from 6-day old cultures grown on potato dextrose agar plates at 30^{o}C were harvested with 0.1% Tween 80 (Difco Laboratories, USA) solution and inoculated into 100 mL medium in 250 mL Erlenmeyer flasks. The basal medium used to develop cultures of *Aspergillus sydowii* designated as medium M was prepared in conical flasks and had the following composition (w/v): 1% beech wood xylan, 0.01% $FeSO_4.7H_2O$, 0.6% K_2HPO_4, 0.2% KH_2PO_4, 0.5% $(NH_4)_2SO_4$. The medium pH was adjusted to 5.6 prior to autoclaving and it was supplemented with 1% (w/v) each of the following carbon sources: carboxy methyl cellulose, xylose, lactose, galactose, glucose, mannitol and sorbitol. Agricultural residues namely rice husk, orange peel, cassava peel, corn cob, corn straw and mango peel were washed in distilled water and boiled in distilled water for 15 min. The water was decanted and the substrates were dried in an oven to constant weight and powdered using a milling machine. The powder was sieved with a mesh sieve and each added into medium M at 1% concentration devoid of xylan. The following nitrogen sources (0.5 % w/v) were each added into medium M in place of $(NH_4)_2SO_4$: sodium nitrate, ammonium hydrogen phosphate, urea, yeast extract, peptone, casein and tryptone. The flasks were autoclaved at 121 ^{o}C for 15 mins and inoculated with 2 x 10^6 spores/mL of *Aspergillus sydowii*. Each flask was incubated at room temperature (30 ±2^{o}C) for 72 h after which the content of the flasks was centrifuged at 2515 x g for 10 min. The supernatant was designated as crude enzyme.

Xylanase assay: Enzyme solution (1 mL) in test tube was incubated with 2 ml of 0.2 M potassium phosphate buffer (pH 5.6) containing 1mL of 0.5% beech wood xylan at 40°C for 60 mins. The amount of xylose formed was determined by a modification of the 3, 5-dinitrosalicylic acid (DNS) method of Miller (1959). Enzyme activity was defined as the amount of enzyme that released 1µg xylose per min under the assay condition.

Partial purification of the enzyme: The crude enzyme was dialyzed overnight against 0.2 M potassium phosphate buffer (pH 5.6). Ammonium sulphate was added to the crude enzyme extract to 45% saturation, incubated for 10 h with gentle mixing. The solution was centrifuged at 2515 x g for 15 min. and the supernatant was subjected to further stepwise precipitation with ammonium sulphate to 60% saturation followed by centrifugation.

The effect of pH on enzyme activity: The effect of pH on activity of the xylanase was determined by using buffer solutions of different pH (citric acid-sodium citrate buffer, pH 2.0-4.0 and potassium phosphate buffer, pH 5.0 to 9.0) for enzyme assay. The pH activity profile of the enzyme was determined by incubating 1 mL of the enzyme contained in test tubes with 1 mL of 0.5 % (w/v) beech wood xylan prepared in 2 mL of buffers of different pH values (2.0-8.0) at 40^{o}C for 1 h. The reaction was stopped by the addition of 2 mL of DNS reagent and enzyme activities were determined.

The effect of temperature on enzyme activity: The effect of temperature on enzyme activity was studied by incubating 1mL of the enzyme solution contained in test tube and 1 mL of 0.5 % (w/v) beech wood xylan prepared in 2 mL of 0.2M potassium phosphate buffer (pH 5.6) for 1 h at various temperatures (20, 25, 30, 35, 40, 45, 50, 55, 60, 65, and 70^{o}C) in a thermo static water bath (Kottermann, Bremen, Germany). The reaction was stopped by the addition of 2 mL DNS reagent and the enzyme activities were determined.

pH stability and Thermal: The pH stability profile of the enzyme was determined by incubating 1 mL of 0.5 % (w/v) beech wood xylan prepared in 2 mL of 0.2 M potassium phosphate buffer (pH 5.6) for 2 h in various buffers at 40^{o}C. The reaction was terminated by the addition of DNS reagent and reducing sugar concentration was assayed at 30^{o}C. The residual activities of the treated enzyme were determined.

Thermal stability was studied by incubating 1mL of the enzyme solution contained in test tube and 1 mL of 0.5 % (w/v) beech wood xylan prepared in 2 mL of 0.2 M potassium phosphate buffer (pH 5.6) at various temperatures (20, 25, 30, 35, 40, 45, 50, 55, 60, 65, and 70^{o}C) in a thermo static water bath (Kottermann, Bremen, Germany) for 2 h. The reaction was terminated by the addition of DNS reagent and reducing sugar concentration was therefore determined at 30 ^{o}C. The residual activity of the treated enzyme was then measured according to the standard assay method.

Analysis: Protein content was estimated by the method of Lowry *et al.*, (1951) using bovine serum albumin (Sigma-Aldrich) as a standard. Reducing sugar concentrations were determined by a modification of the DNS method of Miller (1959). Briefly, DNS (10 g) was dissolved in 200 mL of 0.2 M NaOH. Potassium sodium tartrate (300 g) was dissolved in 800 mL of distilled water. The two solutions were mixed and stored in an air tight dark bottle. Aliquot sample (2 mL) of this reagent was added to tubes containing 1 mL of 1% xylose solution and to distilled water blanks. The tubes were placed in boiling water bath for 15 min. and cooled to room temperature. The solutions were read in a Spectrum Lab 23A spectrophotometer at 540nm. The readings were used to draw a standard curve for micrograms D-xylose per mL against absorbance.

Results and Discussion

Several xylanase – producing isolates were selected after flooding the plates with Congo red solution followed by destaining with sodium chloride solution. All colonies showing a clear zone on agar plates were further screened by growing them in liquid medium and assaying enzyme activity from the cell-free supernatant fluid. One strain designated as *Aspergillus sydowi* produced the best xylanase activity among the isolates tested and was therefore used for further work. *Aspergillus sydowii* selected from 18 isolates clearly had more xylan catabolizing ability than the other isolates.

The fungus utilized all pure carbon sources tested for enzyme production (Table 1) and induced highest level of xylanase production with mannitol (50.1 Unit/mg protein) followed by sorbitol (46.8 Unit/mg protein) after 72 h. Lowest enzyme activities of 12.6 and 10.1 Unit/mg protein were produced with xylose and glucose respectively after 72 h. The use of xylan alone only caused enzyme production of 44.9 Unit/mg protein after 72 h. Tallapragada and Venkatesh (2011) observed that when different carbon sources were used in the production medium for xylanase production, the highest enzyme activity was obtained with oat spelt xylan, while lower activities were obtained with maltose, cellulose, mannitol, sucrose, lactose and dextrose. Glucose inhibited xylanase production. The effect of glucose on xylanase production could be attributed to catabolite repression of enzyme synthesis. Gupta and Kar (2009) observed that xylose addition resulted in lower growth and enzyme yield was perhaps due to the repression of the enzyme synthesis by feed back inhibition as a result of xylan degradation which also produced xylose additionally. Catabolite repression by xylose has been established by various reports (Archana and Satyanarayan, 1997; Lemos and Junior 2002). Under the same reaction conditions, readily metabolizable sugars such as glucose and xylose acted as repressors of xylanase yield (Baitaillon *et al.*, 2000). According to Liu *et al*., (1999) xylose did not cause any catabolite repression of xylanase in the yeast *Trichosporon cutaneum*.

TABLE 1: Effects of carbon sources on the production xylanase by *Aspergillus sydowii*

Carbon source (1 %, w/v)	Period of incubation (h)		
	24	48	72
	Xylanase activity (Unit/mg protein)		
Control (Xylan alone)	12.7	28.3	44.9
Carboxy methyl cellulose	2.3	10.5	14.8
Mannitol	18.9	35.7	50.1
Galactose	7.6	18.4	30.5
Sorbitol	10.2	26.9	46.8
Lactose	8.4	12.3	22.4
Glucose	1.6	5.9	10.1
Xylose	2.8	7.2	12.6

Values are means of duplicate experiments

The xylan of the production medium was replaced with crude agricultural residues namely rice husk, orange peel, cassava peel, corn cob, corn straw and mango peel for enzyme production (Table 2). It was observed that the best enzyme production of 97.8 and 92.6 Unit/mg protein were achieved with orange peel and mango peel respectively. These levels were much higher than that achieved when pure carbon sources or xylan alone were used for enzyme production.

TABLE 2: Production of xylanase by *Aspergillus sydowii* in broths prepared from agricultural residues

Agricultural residue (1 %, w/v)	Period of incubation (h)		
	24	48	72
	Xylanase activity (Unit/mg protein)		
Rice husk	32.4	50.1	71.8
Cassava peel	18.4	28.1	35.1
Orange peel	30.6	72.8	97.8
Corn cob	15.6	30.1	39.2
Corn straw	18.2	25.6	34.9
Mango peel	38.4	61.4	92.6

Values are means of duplicate experiments

The challenges in xylanase production involve developing suitable bioprocess and media for xylan fermentation; besides identification of cheaper substrates and inducers. The use of purified xylan as an inducer increases the cost of enzyme production. Pure xylan is too expensive to be used for industrial production of xylanases. For this reason, different lignocellulosic residues have been used in cultures as inducers for xylanase production (Reis *et al.*, 2003). Therefore alternative substrates, particularly crude agricultural – based raw materials have been explored as potential substrates and inducers of xylanase production (Xu *et al.*, 2005; Sharma and Bajaj 2005).With a view to replace xylan, a costly substrate for xylanase production, various cheap and abundantly available lignocellulosics have been used for xylanase production (Techapu and Prosesor 2003). Agricultural residues have been reported to induce xylanase synthesis efficiently (Bakir *et al.*, 2001; Alves-Prado *et al.*, 2010). Similarly, reports are also available on the positive regulation by lignocellulosic substrates like wheat bran, rice straw, rice bran etc. on the enhanced xylanse production (Beg *et al.*, 2000; Battan *et al.*, 2006). Alves – Prado *et al*., (2010) studied carbon sources including xylan, corn straw, corn cob, wheat bran and sugar cane bagasse for xylanase production. The most significant xylanase production was shown on medium containing corn straw followed by medium containing wheat bran and xylan as carbon sources. Further experiment showed orange pomace as a very efficient carbon source for xylanase production by *Trichoderma harzianum* (Seyis and Aksoz 2005). In another study, xylanase was produced on banana agricultural wastes from *Pleurotus* sp. (Reedy *et al.*, 2003). In a study carried out with *Streptomyces actuosus* A – 151, it was concluded that the addition of orange peel to the cultural medium enhanced the production efficiency of xylanase (Wang *et al.*, 2003). Shanthi and Roymon (2014) reported that inexpensive and abundantly available carbon substrates like wheat bran resulted in good yield of xylanase better than xylan. Bajaj *et al*., (2010) replaced xylan in xylanase production medium with carbon sources namely wheat bran, wheat bran hydrolysate, wheat straw and saw dust and it was observed that *Streptomyces* sp. showed much higher xylanase titre on crude carbon substrates particularly on wheat bran and wheat straw than on xylan. Agricultural based substrates also possess high structural complexity as compared to pure xylan and therefore acted as efficient substrates and inducers of xylanase production (Baitaillon *et al.*, 2000) and in certain cases induced more xylanase production than pure xylan (Bajaj *et al.*, 2010; Baitaillon *et al.*, 2000).

TABLE 3: Effects of nitrogen sources on the production of xylanase by *Aspergillus sydowii*

Nirtogen source (0.5 %, w/v)	Period of incubation (h)		
	24	48	72
	Xylanase activity (Unit/mg protein)		
Organic			
Casein	12.5	20.0	32.4
Tryptone	18.2	21.4	28.3
Yeast extract	17.4	30.8	36.9
Peptone	20.1	32.6	41.2
Inorganic			
Urea	9.0	12.9	20.6
Ammonium hydrogen phosphate	9.8	17.4	25.8
Ammonium sulphate	10.2	20.1	30.4
Sodium nitrate	7.5	15.3	19.9

Values are means of duplicate experiments

The effect of nitrogen sources was tested by replacing $NH_4(SO_4)$ in the enzyme production medium with other nitrogen sources (Table 3). Among various nitrogen sources tested, peptone and yeast extract were found to be the best nitrogen sources for maximum xylanase production with enzyme productivities of 41.2 and 36.9 Unit/mg protein respectively after 72 h. Lowest enzyme yield (19.9 Unit/mg protein) was achieved with sodium nitrate after 72 h. The present study showed that the kind of nitrogen source used in the medium influenced xylanase production. Inorganic nitrogen sources failed to effect good xylanase production. The best nitrogen sources for xylanase production by *Aspergillus niger* was yeast extract; whereas peptone, ammonium sulphate, beef extract, sodium nitrate, meat extract and ammonium nitrate stimulated enzyme production but not as high as yeast extract (Tallapragada and Venkatesh 2011). Organic nitrogen compounds are generally reported as better nitrogen sources for xylanase production by microorganisms (Kuhad *et al.*, 2006). Shanthi and Roymon (2014) tested various nitrogen sources, including organic and inorganic to determine the best nitrogen source supporting maximum xylanase production. Nine different nitrogen sources were examined of which the highest xylanase production was observed in medium containing yeast extract and peptone. Yeast extract and peptone have been reported to play an important role in xylanase production because of the presence of essential elements and growth factors necessary for the growth of organisms (Porsuk *et al.*, 2013). Peptone is a complex organic nitrogen source which might be stimulating growth by releasing NH_4^+ and improving the expression of nitrogen assimilating enzymes (Wang *et al.*, 2009).

Enzyme activity was assayed in the presence of different range of buffers (Fig 1). The enzyme exhibited a broad pH profile (from 2.0 – 9.0). The optimum pH for the activity of xylanase was 4.0 and enzyme activity decreased remarkably thereafter. The xylanase pH stability optimum was at 3.0-5.0 and only 51 and 22% of the maximum activity remained at pH 8.0 and 9.0 respectively (Fig 2).

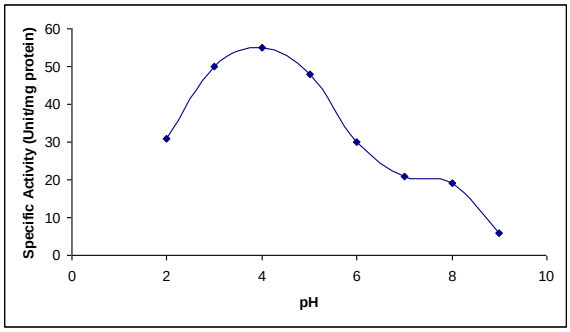

Figure 1: Effects of pH on enzyme activity

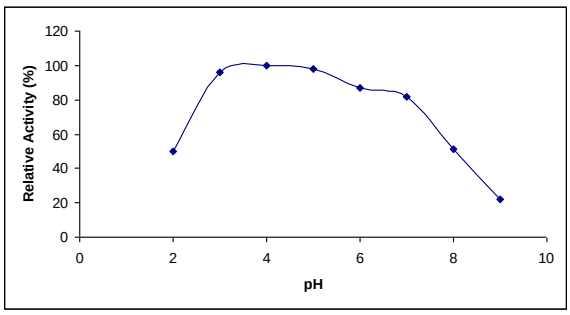

Figure 2: Effects of pH on enzyme stability

Xylanase exhibited optimum pH activity and stability ranges of 4.0 and 5.0 for *Aspergillus aculeatus* (Fujimoto *et al.*, 1995); 8.0 for *Aspergillus nidulans* KK – 99 (Taneja *et al.*, 2002); 5.0 for *Aspergillus oryzae* (Kitamoto *et al.*, 1999); 4.0 for *Aspergillus sydowii* (Ghosh and Nanda 1994); 6.0 for *Bacillus* sp. (Baitaillon *et al.*, 2000); 3.8 for *Penicillium capsulatum* (Ryan *et al.*, 2003); 2.0 for *Penicillium* sp. (Kimura *et al.*, 2000). pH stability of 4.0 – 5.5 for *Aspergillus awamori* (Kormelink *et al.*, 1993); 5.0 – 7.0 for *Chaetomium cellulolyticum* (Baraznenok *et al.*, 1999); 6.0 – 8.0 (Georis *et al.*, 2000) for *Streptomyces* sp; 4.0 – 5.0 for *Thermoascus aurantiacus* (Kalogeris *et al.*, 1998); 6.0 – 6.5 for *Thermomyces lanuginosus* (Singh *et al.*, 2000) have been reported.

The enzyme temperature optimum was at 35°C. At 20°C a very low titre of xylanase was recorded (Fig 3). Optimum thermostability was observed at 30-

40ºC (Fig 4). Temperatures beyond this range were not found suitable for enzyme stability. These characteristics were compared to those reported for xylanases from other microorganisms. Optimum xylanase production was reported at 37ºC (Battan *et al.*, 2006); 48ºC for *Penicillium capsulatum* (Ryan *et al.*, 2003); 50ºC for *Aspergilllus sydowii* (Ghosh and Nanda 1994); 55ºC for *Aspergillus nidulans* KK – 99 (Taneja *et al.*, 2002); 60ºC for *Aspergillus oryzae* (Kitamoto *et al.*, 1999); 75ºC for *Bacillus* sp. (Baitaillon *et al.*, 2000); 70ºC for *Thermomyces lanuginosus* (Singh *et al.*, 2000). Temperature stability of 55 – 60ºC for *Streptomyces* sp. (Georis *et al.*, 2000); 70 – 75 ºC for *Thermoascus aurantiacus* (Kalogeris *et al.*, 1998) and 45 – 55 ºC for *Aspergillus awamori* (Kormelink *et al.*, 1993).

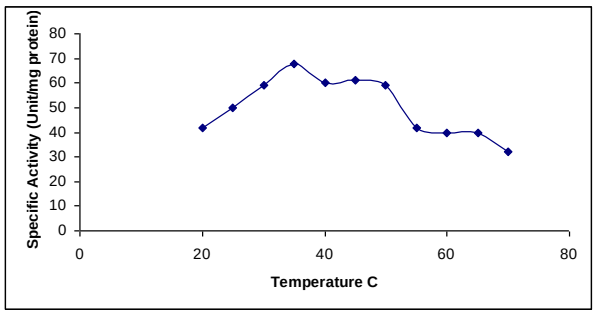

Figure 3: Effects of temperature on enzyme activity

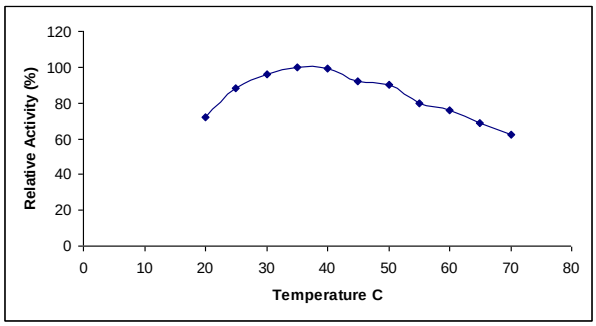

Figure 4: Effects of temperature on enzyme stability

Conclusion

Aspergillus sydowii was the most potent one from 18 isolates and was identified by xylano hydrolytic zones on xylan agar plates. The influence of carbon substrates on enzyme production showed that the medium containing mannitol produced a maximum enzyme yield of 50.1 U/mg protein after incubation for 72 h. The enzyme production in this medium was much higher in a medium that contained only xylan (44.9 U/mg protein) as the sole carbon source. Xylose and glucose were poor carbon substrates for xylanase production. Peptone and yeast extract were the best nitrogen sources for enzyme production. The enzyme showed maximum activity (55 U/mg protein) at pH 4.0 and optimum stability at pH 3.0-5.0. Temperature activity profile of the enzyme showed best activity (68 U/mg protein) at 35ºC. Best temperature stability occurred at 30-40 ºC. Dried agricultural residues were tested for their abilities to support xylanase production in media devoid of xylan. The best enzyme productivities of 97.8 and 92.6 U/mg protein were achieved after 72 h in the medium containing orange peel and mango peel respectively. These levels were much higher than that achieved when pure carbon sources or xylan alone were used for enzyme production.

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