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Characterization and partial purification studies on α-amylase activity by *Fusarium verticillioides*

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Abstract

The intention of present study was to optimize the production and partial purification of α-amylase *Fusarium verticillioides*. The source was provide and cultured in the media, prepared by cheap agricultural substrates used in liquid broth culture, the produced α-amylase which was interfere with complex carbohydrate digestion. The production of enzyme aimed to discover a suitable extraction method to hydrolysis starch from different carbohydrate source. *Fusarium verticillioides* was produced α-amylase from less expensive agricultural yields likes carbohydrate containing vegetables. Fermentation in 250ml Erlenmeyer flasks, process carried out in shaker 250rpm. Different cultural conditions effect the different concentration of nitrogen source and also it influenced by micro element solution. The maximum production obtained in maize starch, and optimum temperature for growth at 30ºC at pH7.0 was found to be the optimum for the synthesis of the enzyme. The temperature influence the incubation condition was studied by different growing temperatures between 20ºC to 37ºC. Fermentation carried out using sweet potato, potato, maize, maize cob powder and tapioca starch in different concentrations used in basal medium. 5% of starch is for maximum yield. Organic and inorganic source yeast and sodium acetate found to be the best for production. 50ml of media in 250ml of Erlenmeyer flask is best for aeration. Partial purification of enzyme was active in wide range of values in pH 3-9 optimum activity exhibited at pH 7.0 and temperature 30ºC. The enzyme activity gradually decreases in rising of temperature. The enzyme activity completely lost in 15 minutes of incubation at 100 ºC.

Key words: α- amylases, *Fusarium verticillioides,* enzyme purification & influence of metal ion

Critical survey of the available literature indicates that Fusarium verticillioides is the fungus most frequently found in corn kernels (Gonzlez el al., 1995). It is responsible for production of a group of structurally related metabolites known as fumonisins. *Fusarium verticillioides* (Sacc.) Nirenberg (syn. *F. moniliforme* J. Sheld.) is the most

prevalent fungus associated with maize (*Zea mays* L.) (Marasas, 2001., Miller, 2001 and Nelson, 1992), The present study dealing with biochemical activities of *Fusarium* are those which deals with the production of exo-polysaccharides (Stasinopoulos and Seviour, 1989) and β-glucanases (Pitson et al., 1991,1997). The factors influencing the synthesis of the enzyme are presented. Amylases are a group of enzymes that break down starch or glycogen. They are produced by a variety of living organisms including fungi, bacteria (*Aspergillus niger*, *Aspergillus fumigates, Tribolium castaneum and Callosobruchus maculatus*). (Fodel, 2000; Yabuki et al., 1977; Young et al., 2001) and also higher plants. The natural occurrence of *Aspergillus oryzae* alpha amylase (Blanco L A and Iturbe C,1981). Later, Mahoney et al. (Mahoney et al., 1984) and BlancoLabra et al. (Blanco L A et al., 1995) characterize the protein activity. These findings triggered interest in the study of the Microorganisms synthesize and release amylases extracellularly. Amylases are classified on the break down pattern of starch molecule.

α-Amylases (endol-1-4-α-D-glucan glucohydrolase, EC 3.2.1.1) reduce the viscosity of starch by breaking down 1-4-α-D-glucosidic bonds at random in the linear amylase chain and thus producing varied sized chains of glucose. These are endo enzymes that split the substrate in the interior of the molecules and are classified according in their action and properties. The families of amylase enzymes are of great significance due to its areas of potential applications in higher fructose corn syrup preparations, additives to detergents to remove strains, saccharification of starch for alcohol production and brewing, fermented drink industry and in the production of adhesives etc. Interestingly the major advantages of using microorganisms for production of amylases includes economical bulk production capacity and easy manipulation of microbes obtain enzymes of desired characteristics (Panday, 2003).

Amylases find potential application in a number of industrial processes like food, textiles and paper industries. Microbial amylases have successfully replaced chemical hydrolysis of starch in starch processing industries. They would be potentially useful in pharmaceutical and fine chemical industries if enzyme with suitable properties could be prepared (Fogarty, 1980 and Kelley et al., 1997). Emergence of biotechnology offered new avenues of amylase application in many other fields like bio-pesticides, aroma compounds, biopharmaceuticals and other bioactive compounds, (Ogbonna CN et al., 2014) Recently Witczak (1999) presented a review of thio-sugars as potential new therapeutics, which are gaining substantial attention.

The other advantage of using filamentous fungi is that the fungal mycelia synthesize and release large quantity of extra-cellular hydrolytic enzymes (Manpreet et al., 2005; Kim et al., 2003). Among a large number of non- pathogenic microorganisms capable of producing useful enzymes are particularly interesting due to their easy cultivation, and high production of extracellular enzymes with potential industrial exploitation. The use of starch degrading enzymes was in fact the first large-scale application of microbial enzymes in the food industry (Pandey et al., 2000; Abe et al., 1988). There are several processes in medical areas that involve the application of amylases (Sutton et al., 1999; Giri et al., 1990; Ch et al., 1997; Stanberg et al., 1999). α-Amylase is useful as thermistor for the biochemical analysis of cyclodextrins (Kolb et al., 1996).

In the recent years genetic engineering has been used extensively for cloning of amylase producing strains mainly α-amylase and glucoamylase with the view to achieve desirable production levels in the cloned host in addition to co-expression of two enzymes by the same organism. A great deal of work has been done on cloning α-amylase producing strains more commonly employing *Saccharomyces cerevisiae* or *E. coli* and Aspergillus niger (Liebl et al., 1997; Birol et al., 1998; Abe et al., 1988; abu et al., 2005). The aim of the present work was the indigenous production of *Fusarium verticillioides* by using different sources of the starch material and the effect of different process parameters are studied for growth and maximum yield of enzyme and its activity and partial purification

MATERIALS AND METHODS

Sample collection

Samples were collected from different source from soil, under sterile conditions. The isolation of fungi was carried under pour plating method, this process were repeatedly inoculated in petri dish containing PDA media (Fermentation medium comprising of starch 20 g/L, yeast extract 0.5 g/L, KH2PO 10 g/L, (NH4)2SO 10.5 g/L, MgSO4.7H4O 0.3 g/L, CaCl2 0.5 g/L, FeSO4.7HO trace element, MnSO4.7H2O 0.004 g/L and ZnSO4.H2O and trace element solution)**.** This process was repeated until to get pure culture without any contamination. Along with strains were placed at various temperatures like 4ºC to 37 ºC to find out optimum temperature for healthy growth.

Seed development:

Among the characteristics of suitable conditions we observed colonial characteristics such as surface appearance, texture and color of colonies, along with that microscopy observations like vegetative mycelium with or without cross walls, diameter of hyphae and spore size range for taxonomic descriptions(David et al., 2007). Fermentation process dealing with secondary metabolites, amylase production by *F. verticillioides* was carried out as biphasic system i.e. development of seed followed by fermentation process. These steps are essential because environmental factors required for optimum growth and reproduction of the strain might differ from those required for the production of the enzyme. These parameters include carbon, nitrogen, source, pH of the medium, temperature of incubation, aeration and inoculum concentration.

Fermentation and amylolytic activity

A well grown, heavily sporulating culture slant was selected and 5 ml sterile distilled water was aseptically added. With the help of a sterile inoculation needle the spores were carefully scraped off and the whole suspension was added to 250 ml Erlenmeyer flask containing 75 ml sterile distilled water. 0.5 ml of this, was used to inoculate 50 ml sterile seed medium present in 250 ml Erlenmeyer flask 2.0 ml of fresh, healthy

and actively growing seed was used to inoculate 250 ml Erlenmeyer flasks containing 50 ml fermentation medium. The composition of the fermentation medium is same as the seed medium except it contained microelement solution. Triplicates were used for each experiment. The culture was grown as before under submerged conditions on reciprocating shaker for 5-6 days. At the end of every 24 hrs samples were collected to determine pH, growth pattern and purity of the culture and enzyme activity. At the end of 144 hrs. of incubation, the contents of all the flasks were pooled; pH determined using a pH meter and mycelial mat was separated by filtration through Whattman number 1 filter paper fixed in a Buchner funnel. The filtrate was collected for enzyme estimation.

α-Amylase activity was assayed by the method of McMahon et al (1977) with slight modification of optimum temperature and pH. One unit of amylase activity (μmol/min) was defined as the activity of 1 ml of enzyme solution that produced 1μmol of reducing sugar per minute from starch at pH 7.0 and 30^{o}C. Reducing sugar was determined by the 3,5-dinitrosalicylic acid (DNS) method (Samarntarn and Tanticharoen, 1999) using glucose as a standard. The fermentation broth contained some suspended particles, which interfered with the determination of cell weight by filtration and drying. Thus protein content was used instead of dry mass for evaluation of cell growth. Protein contents were determined by the standard Kjeldahl method (Kjeldahl, 1983). All the fermentation experiments and enzyme assay were carried out in triplicate with analytical grade reagents and the mean values were reported.

Partial Purification of α-amylases

The crude enzyme extract was examining though ammonium sulfate. The properties of the enzyme and activity of amylase was partially purified. A precipitation test was conducted using ammonium sulphate. After salting out with ammonium sulfate ranging from 30 to 80 % saturation, recovery of α-Amylase activity was below 50 % under all test conditions. The ammonium sulfate precipitation of the proteins went through addition of salt in a range between 60% and 100% saturation. On the basis of these results, salting out with 60 % ammonium sulfate was the most effective from the perspective of recovery and specific activity of α-Amylase. After centrifugation at 7500g for 15min and dialysis for 24hrs (10kdm dialysis membrane used), with phosphate buffer solution

The purity of extract was eluted on sephadex G -100 column using 50mM phosphate buffer Ph 7.0. at flow rate 10ml/hrs. Each 3ml of fractions were collected and stored in ice, followed by amylase assay. After getting the result of each fraction enzyme activity was found with a range of pH 6 – 8. Optimum activity was exhibited at ph 7.0 and temperature 70^{o}C. The enzyme became inactive at 100^{o}C in 15 min incubation. The fractions of highest amylase activity were kept at 4^{o}c for further analysis.

RESULTS

The influence and maximum production of amylase was shown in following aspects. Regarding to the Hydrogen ion concentration exerts tremendous influence on carbon

nitrogen metabolism, growth, sporulation biosynthesis and release of metabolites by microorganisms. Therefore the effect of hydrogen ion concentration on the synthesis of α- amylase by *Fusarium verticillioides* was investigated by inoculating buffered nutrient medium having different pH values. 2 % spore inoculum was used to inoculate 50 ml of sterile medium in 250 ml Erlenmeyer flasks (Fig 10). At the end of the growth cycle final pH of each was noted. At acidic pH 3 culture growth was poor and enzyme activity was negligible. Increased biomass and enzyme production were made at range of pH 3 to 9 (Fig.1). Achieved maximum or near maximum growth in the pH range 5.0 to 8.0 But best growth of enzyme occurred at initial pH of the fermentation was adjusted to pH 7. During the fermentation pH was noticed in different levels. But end of the fermentation process it reached to pH 8.7, if the fermentation process started at alkaline pH in range of 8 to 10 the activity of the enzyme decreased drastically. All the experiments initial pH were taken as 7.0 Temperature influence also noticed between environment and microorganism growth and it constitutes one of the essential parameters for the metabolic process to proceed effectively. Therefore influence of incubation temperature was studied by growing the culture at different temperature like 20 ºC, 30 ºC, 35 ºC and 37 ºC. low activity of enzyme was at 20 ºC and below. The pH of the culture broth at the end of fermentation remained near neutrality with increase in temperature to 30 ºC (Fig 2). There has been steadily increased the mycelial growth and synthesis of the enzyme At 144 hrs. of the fermentation. Maximum synthesis of enzyme at 30ºC and it is noticed as optimum temperature gradually the color of the broth was also developed relatively deep blue black alkaline pH and biomass also increased. Increasing temperature was not given any improvement on production of amylase.

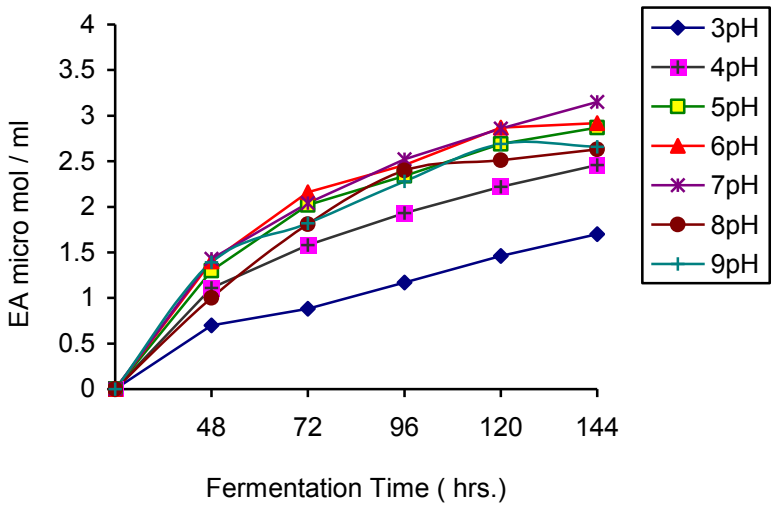

Fig. 1: Effect of pH on the synthesis of α-amylase by *Fusarium verticillioides*

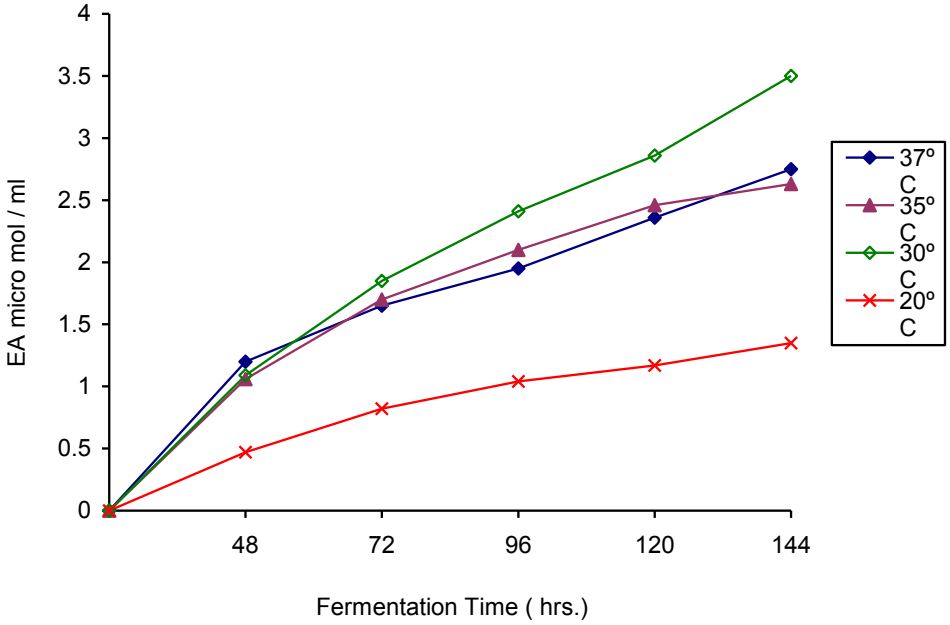

Fig. 2 Effect of Temperature on the synthesis of α-amylase By *Fusarium verticillioides*

Effect of carbon source

The present investigation in order to determine the best source of carbon which promote growth and synthesis of the enzyme was maize starch. It provided the maximum synthesis of amylase activity and high yield of biomass was found. Microscopic observation the mycelium appeared healthy without vacuoles. Potato starch was found the next best source of carbon. Maize cob powder, sweet potato powder, tapioca starch and rice starch are less preferred sources of carbon and stand in descending order of preference for the synthesis of the enzyme(Fig 3). By the determination of optimum concentration of maize starch at 7.5% was found the best for optimum synthesis of enzyme 3.36 µmol/ml (Fig 4). While increase or decrease of the carbon source to optimum, amylase activity was gradually decreased. Therefore 7.5% maize starch used as optimum for all experiments. Addition of small quantities of glucose in the initial stages of fermentation did not show any stimulatory effect as claimed for *Aspergillus ochraceus* producing α-Amylase (Ely and Waldemarin, 2002).

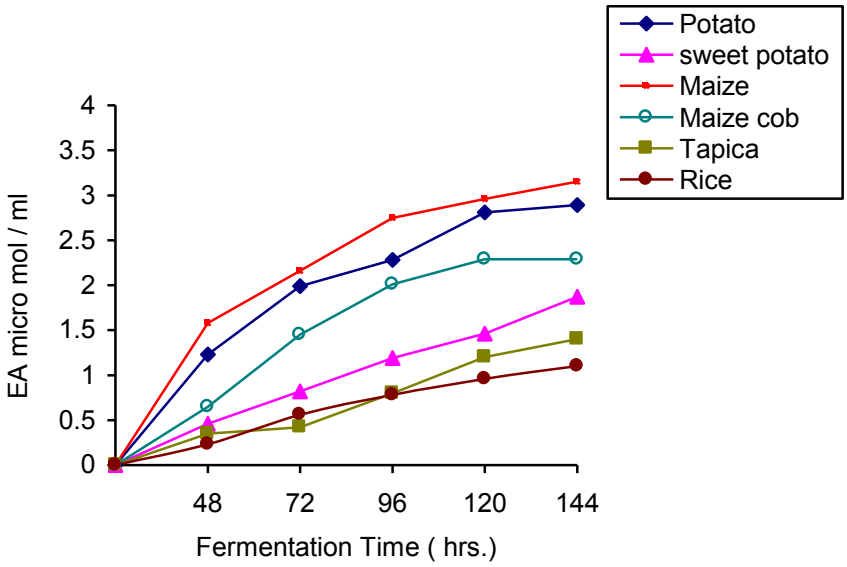

Fig. 3. Effect of different carbon substances on the synthesis of α- amylase by *Fusarium verticillioides*

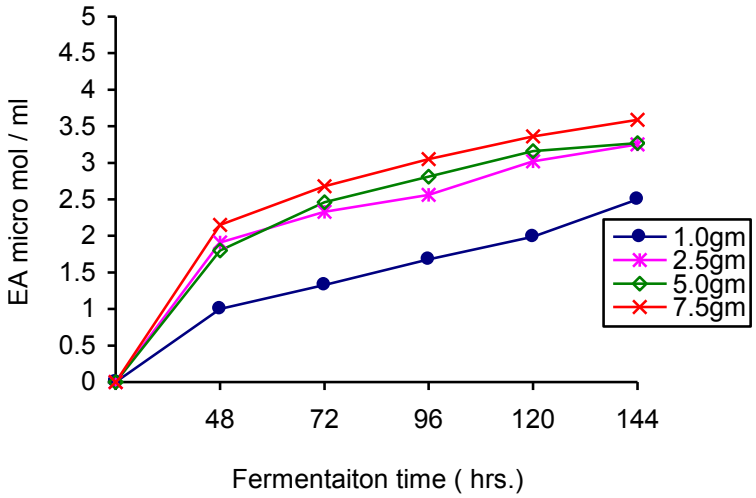

Fig. 4. Effect of different concentrations of Maize starch on the synthesis of α-amylase by *Fusarium verticillioides*

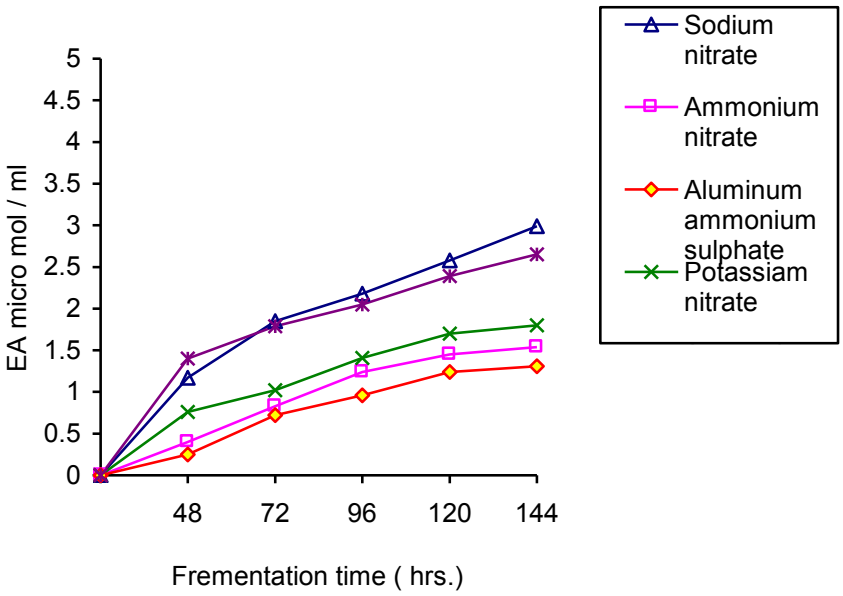

Fig. 5. Effect of different inorganic compounds on the synthesis of α- amylase by *Fusarium verticillioides*

Effect of nitrogen source

Various organic and inorganic nitrogen source incorporated in the basal medium and their effect on the synthesis of amylase have been studied (Fig 5). In all the inorganic nitrogen sources sodium nitrate was found be the best inorganic source of nitrogen for synthesis the enzyme. Increase in sodium nitrate concentration from 0.1-0.5 % progressively increased the enzyme activity 3.25 µmol/ml (Fig 6). Further increase in sodium nitrate concentration did not help to increase the enzyme synthesis. Ammonium sulphate was found next best source of nitrogen but inferior to sodium nitrate. Low activities of the enzyme were observed in the presence of potassium nitrate 1.8 µmol/ml, aluminum ammonium sulfate 1.31 µmol/ml. While fermentation mycelium was observed In aluminum ammonium sulphate the mycelium poorly developed with large vacuoles. In organic source yeast found to be best 2.7 µmol/ml (Fig 8) and followed by peptone soyha been meal (Fig 7). Urea was noticed as poor development in culture growth condition.

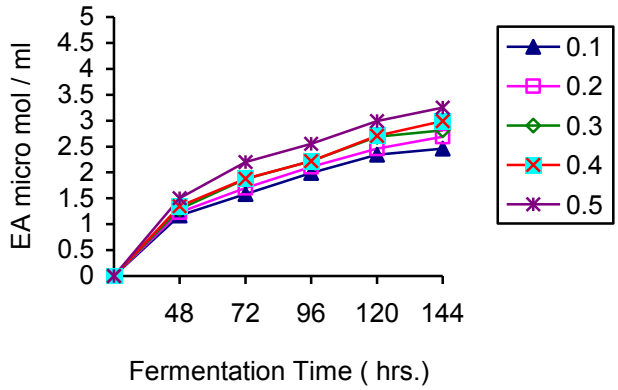

Fig. 6. Effect of different concentrations of sodium nitrate on the synthesis of α-amylase by *Fusarium verticillioides*

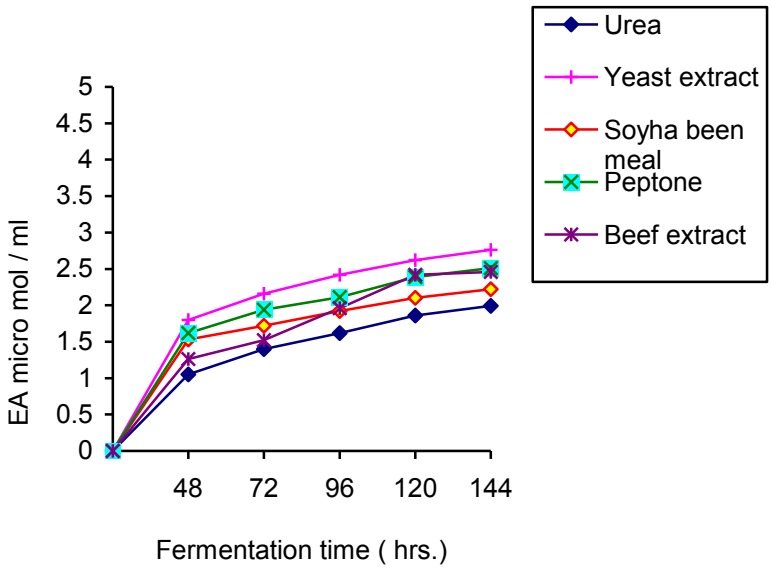

Fig. 7. Effect of different organic sources of nitrogen on the synthesis of α-amylase by *Fusarium verticillioides*

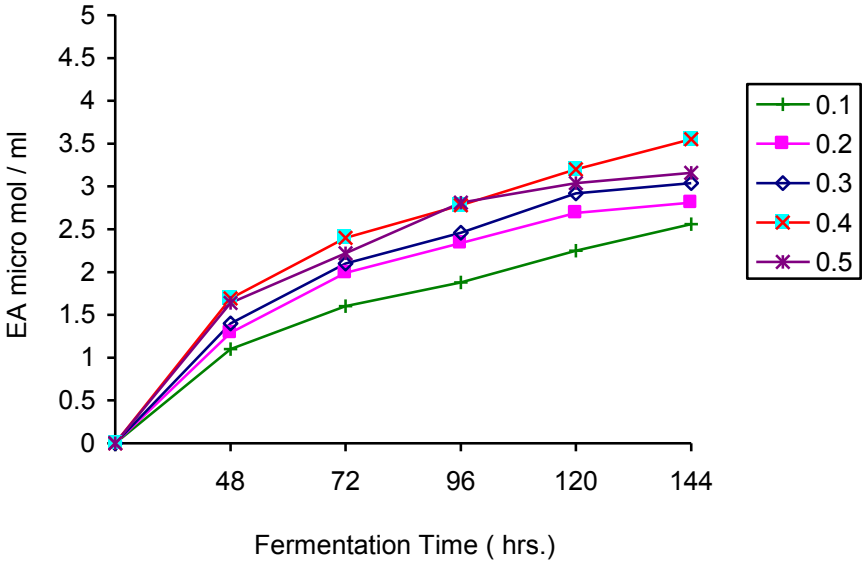

Fig. 8. Effect at different concentrations of yeast extract on the synthesis of α-amylase by *Fusarium verticillioides*

Effect of innoculum concentration

Different size of innoculum range from 0.5ml to 1.5ml was evaluated at pre optimized conditions for maximum yield of cell biomass. All the experiments conducted in triplicate by changing individual parameter. Because quality, purity and age are some of the important factors that affect the final synthesis of the fermentation product. The strain must retain its product forming capacity even after repeated transfers. The seed was grown as before for 72 hrs. and at the end of which sample was aseptically drawn to determine packed cell volume pH, sterility both by microscopic examination and also by inoculation of a loop-full of the seed in a sterile nutrient solution and incubation at 37ºC temperature. When the seed was found pure, and in required state of growth it was used to inoculate 50 ml sterile fermentation medium present 250 ml Erlenmeyer flasks. The inoculum was used in 0.5 ml to 2.5 ml quantities with 0.5 ml instruments. At 2.0 ml concentration of the inoculum and above there was gradually decrease in the enzyme activity in range of 3.45 µmol/mlt to 2.3 µmol/ml (Fig 9). Therefore 1.5 ml inoculum was found optimum for the maximum yield of the enzyme

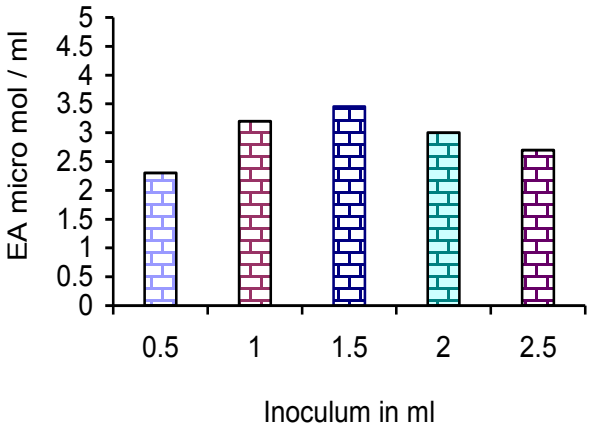

Fig. 9. Effect of inoculum concentration on the synthesis of α-amylase by *Fusarium verticillioides*

Effect of microelement solution

The composition of the basal medium for *Fusarium verticillioides* biomass cultivation was carried with various trace elements (Fig 11). The effect of trace elements, 0.1 ml of trace elements solution was added to each flask of containing 50 ml sterile fermentation medium in 250 ml Erlenmeyer flasks. Simultaneously the individual components of the trace elements solution were separately added to each flask containing 50 ml fermentation medium. Triplicates were used for each element. In comparison with other Fe $(NH_3)_2$ SO_3 is found essential for the enzyme synthesis. Addition of complete trace element solution has contributory role in enhancing enzyme activity.

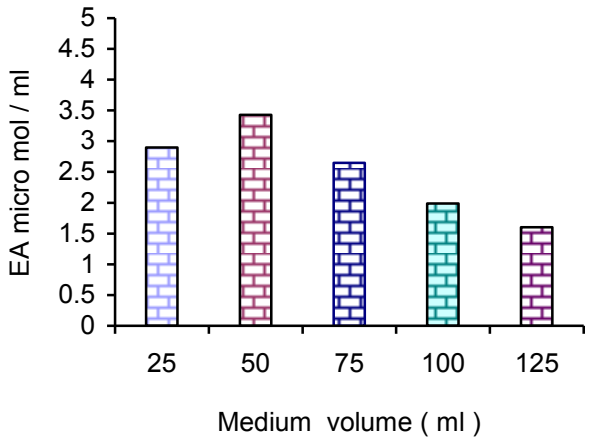

Fig. 10. Effect of aeration on the synthesis of α-amylase by *Fusarium verticillioides*

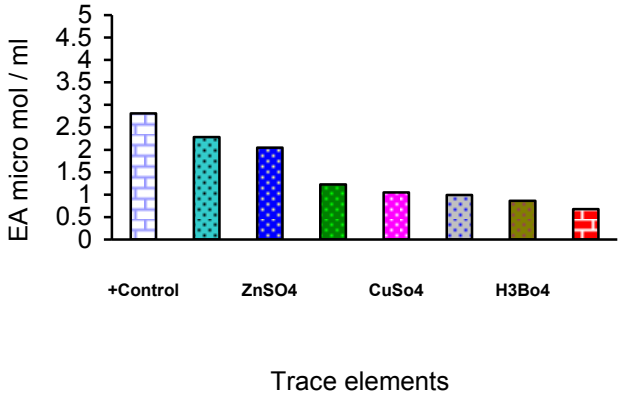

Fig. 11. Effect of trace element solution on the synthesis of α-amylase by *Fusarium verticillioides*

Partial purification and characterization

Partial purification of crude enzyme extract carried though salting out process. The enzyme assay was performed to determine the amylase activity in ammonium sulfate fractions. Separations of was seen in 30% to 70% fractions of supernatant obtained. Amylase obtained from 72hrs of fermentation process to isolate the amylase was carried in 8% of SDS PAGE gel electrophoresis

DISCUSSION

The most active fungal isolate was identified from soil and the cultural and morphological character and 18s rDNA sequence, was identified as *Fusarium verticillioides.* The activity of amylase was carried out by DNS method. The amylase activity in the fungal supernatant was 3.15 µmol / ml at 30°C. The enzyme activity after precipitation by ammonium sulphate and DEAE sephadex purification was 3.5 µmol /ml. and 4.16 µmol /ml respectively. These results reflect some loss in the enzyme during purification, even though the specific activity was increased after the sephadex purification. The maximum amylase activity was observed at pH 7.0. Therefore, we recommend the use of this enzyme in commercial application aspects like detergents. Further increase in the incubation temperature in habited the enzyme. Stability against organic solvents is one of important when using an enzyme in industrial applications. Ammonium sulphate was found best source of nitrogen. Comparatively organic source was yeast. During the fermentation process growth of mycelium noticed including well without vacuoles and poor with vacuoles. Innoculum size 1.5ml inoculums in 250ml Erlenmeyer flasks was found as optimum for maximum yield of the enzyme. Role of trace elements in biomass cultivation was noticed Fe $(NH_3)_2\,SO_3$ was found essential for the enzyme synthesis.

The specific activity of amylase was 3.5 µ mol /ml at 30ºC. In partial purification 3fold increase in specific activity compared to crude. The specific activity of amylase was 4.16 µmol /ml at 30ºC. The observation was noted in polyacrylamidegel electrophoresis. Molecular weight of amylase from *Fusarium verticillioides* ranges between 50kDa to 60kDa and approximately 54KDa (Fig.12), though some exception exists in case of amylase from *Bacillus licheniformis* (mol. Wt 31kDa) (Raul et al., 2014). High market demand of amylases with specific application in the food and pharmaceutical industries necessitate production and partial purification of this valuable enzyme using cheap row material like maize starch and maize cob. With this goal the present work is carried out to purify amylase from *Fusarium verticillioides* can be used in number of areas like detergent, textiles, hydrolysis of oil field drilling fluids, and paper industry. And also fermentation has emerged as a potential technology for the production of microbial products such as enzymes, feed, fuel, food, industrial chemicals and pharmaceutical products.

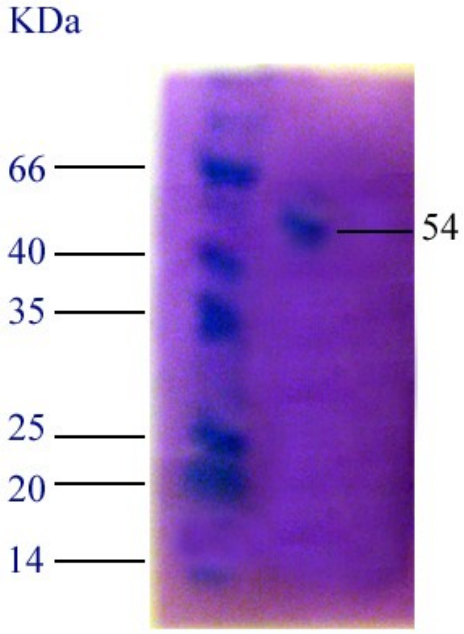

Fig.12. SDS PAGE showing the molecular weight of purified α-amylase on protein marker

CONCLUSION

In summary of the amylase production and its interesting properties in vitro that could of interest, particularly for possible effect on the culture condition and maximum yield enzyme activity (µmol/ml) and specific activity as compared to amylase produced from *Fusarium verticillioides* its amylolytic properties. We also observed that specific activity of increased by saturation of enzyme by ammonium sulphate precipitation. Purification of enzyme was obtained after dialysis with the increase in

the specific activity. Thus we conclude the *Fusarium verticillioides* can produce sufficient amount of amylase having a good economic and industrial application.

REFERENCES

[1] Abe J, Bergmann FW, Obeta K, Hizukuri S (1988). Production of the raw starch digesting amylase of *Aspergillus* sp. K-27. Appl. Microbiol. Biotechnol. 27:447-450.

[2] Abu EA, Ado SA, James DB (2005). Raw starch degrading amylase production by mixed culture of *Aspergillus niger* and *Saccharomyces cerevisae* grown on *Sorghum pomace*. Afr. J. Biotechnol. 4:785-790.

[3] Birol, G., Onsan, Z.I., Kirdar, B and S. G, Oliver. 1998. Enzyme.Microbiol. Technol. 22: 672-677.

[4] Blanco-Labra, A., and Iturbe-Chi◌as, F. A. 1981. Purification and characterization of an -amylase inhibitor from maize (*Zea maize*). J. Food Biochem. 5:1-17.

[5] Blanco-Labra, A., Chagolla-Lopez, A., Martnez-Gallardo, N., and Valdes-Rodriguez, S. 1995. Further characterization of the 12 kDa protease/ alpha-amylase inhibitor present in maize seeds. J. Food Biochem. 19:27-41.

[6] Ch, G.J., Moon, I. S and J. S. Lee. 1997. Chem. Lett. 6:577-578 cf. Pandey A., Poonam.N., Carlos. R., Vanete. T. Soccol., Dalel Singh and Radjiskumar Mohan. 2000. Advances in microbial amylases (Review). Biotechnol. App. Biochem. 31: 135-152

[7] David S Hibbett, Manfred Binder, Joseph F. Bichoff and his colleagues. 2007. A higher-level phylogenetic classification of the fungi. The British mycological society. 111: 509-547

[8] Fadel-N 2000. Production of thermostable amylotytic enzymes by *Aspergillus niger* F –909 under solid state fermentation. Egyption Jour. Microbiol. 35: 487- 505.

[9] Giri, N. Y., Mohan, A. R., Rao, L. V and C. P. Rao. 1990. Curr. Sci. 59:1339-1340. cf. Pandey A., Poonam.N., Carlos. R., Vanete. T. Soccol., Dalel Singh and Radjiskumar Mohan. 2000. Advances in microbial amylases (Review). Biotechnol. App. Biochem. 31: 135-152.

[10] Gonzlez, H. H. L., Resnik, S. L., Boca, R. T., and Marasas, W. F. O. 1995. Mycoflora of Argentinean corn harvested in the main production area in 1990. Mycopathologia 130:29-36.

[11] Kedera, C. J., Plattner, R. D., and Desjardins, A. E. 1999. Incidence of *Fusarium* spp. and levels of fumonisin B1 in maize in western Kenya. Appl. Environ. Microbiol. 65:41-44.

[12] Kim SW, Hwang HJ, Xu CP, Sung GH, Choi JW, Yun JW (2003). Optimization of submerged culture process for the production of mycelial

biomass and exo-polysaccharides by *Cordyceps militaris* C738. J. Appl. Microbiol. 94:120-126.

[13] Kjeldahl. J. 1983. A new method for the determination of nitrogen in organic matter. Z. Aal. Chem., 22:236.

[14] Kolb, M.., Zentgraf, B., Mattisson, B., Arividson, P and B, Denielsson, 1996. Biochemical–analysis of cyclodextrins using an enzyme thermistor. Thermochim. Acta.227: 1-6.

[15] Liebl, W., Stemplinger, I., and P.Ruile 1997. Properties and gene structure of the thermotaga maritina α-amylase A my A, a putative lipoprotein of a Hyperthermophilic bacterium. Bacterial. 179: 941-948.

[16] Mahoney, W. C., Hermodson, M. A., Jones, B., Powers, D. D., Corfman, R. S., and Reeck, G. R. 1984. Amino acid sequence and secondary structural analysis of the corn inhibitor of trypsin and activated Hageman Factor. J. Biol. Chem. 259:8412-8416

[17] Manpreet S, Sawraj S, Sachin D, Pankaj S, Banerjee UC (2005). Influence of ProcessParameters on the Production of Metabolites in Solid-State Fermentation. Malay. J. Microbiol. 1(2):1-9.

[18] Marasas, W. F. O. 2001. Discovery and occurrence of the fumonisins: A historical perspective. Environ. Health Perspect. 109 (Suppl. 2):239-243.

[19] McMahon, H. E. M., Kelly, C. T., and Fogarty, W. M 1997. Effect of growth rate on α-amylase production by *Streptomyces* sp. IMD 2679. Appl. Microbiol. Biotecnol. Biotechnol., 48: 504-509.

[20] Miller, D. J. 2001. Factors that affect the occurrence of fumonisins. Environ. Health Perspect. 109(Suppl. 2):321-324.

[21] Nelson, P. E. 1992.Taxonomy and biology of *Fusarium moniliforme*. Mycopathologia 117:29-36.

[22] Ogbonna C. N, Okpokwn N. M, Okafor. C. U, and Onyia C. E. 2014. Isolation and screening of amylase producing fungi obtained from garri processing sit. International journal of biotechnology and food science. Bol, 2(5) pp. 88-93.

[23] Pandey A, Soccol CR, Mitchell D (2000). New developments in solid-state fermentation: bioprocesses and products. Proc. Biochem. 35:1153-1169.

[24] Pitson, S. M, Seviour, R.J ., Bolt, J and S.J. Stasinopoulos 1991 Production and regulation of β-glucanases in *Acremonium* and *Cephalosporium* isolates My col. Resh. 95: 352-356.

[25] Pitson. S.M, Robert, J. Seviour, and Barbara, M. Mcdougall. 1997. Effect of carbon source on extracellular (1-3) and (1-6)-β-glucanase production *Acremonium persicinum*. Can. Jour. Microbial. 43: 432-439.

[26] Raul D, Biswas T, Mukhaopadhyay S, Kumar Das S, Gupta S. 2014. Production and partial purification of alpha amylase from bacillus subtilis

(MTCC 121) using solid state fermentation. Biochemistry Research international. pp 1-5

[27] Samarntarn, W., and Tanticharoen, M. 1999. Alkaline protease of a genetically engineered *Aspergillus oryzae* for the use as a silver recovery agent form used X- ray film. J. Microbiol. Biotechonl., 9: 568-571.

[28] Stanberg, A., Nystrom, A., Behr, S. and., A Karisson 1999. Chromatography. 50: 215-222. cf. Pandey A., Poonam.N., Carlos. R., Vanete. T. Soccol., Dalel Singh and Radjiskumar Mohan. 2000. Advances in microbial amylases (Review). Biotechnol. App. Biochem. 31: 135-152

[29] Stasinopoulos, S.J and R.J. Seviour 1989 Exopoly saccharides formation by the isolates of *Cephalosporium* and *Acremonium*. Mycol. Resh. 92: 55-60.

[30] Sulton, A..,Dawson,H., Hoff, B., Grift. E and M.Shoukri. 1999. Analyte comparisons between clinical chemistry analyzers. Can Vet. Jour.40: 225-260.

[31] Yabuki, M., N. Ono., K. Hoshino and S. Fukui 1977 Rapid indiction of α - smylase by non growing mycelia of *Aspergillus oryzae* – Appl. Environ. Microbiol. 34: 1-6.

[32] Young, M. H., Gun, L. D., Hoon, Y. J., Ha P. Y and K. Y. Jae. 2001. Rapid and simple purification of a novel extracellular beta-amylase from *Bacillus sp.* Biotech. Letters 23: 1435 – 1438.