Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 5, Issue, 04, pp.748-752, April, 2013

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

BIOPROCESSING OF *Curcuma angustifolia* FOR ALPHA-AMYLASE PRODUCTION BY *Aspergillus niger*

Rajila, C., Liji, T., Rinku Sundar and *Suganyadevi, P.

Department of Biotechnology, Nallamuthu Gounder Mahalingam College, Pollachi, Coimbatore, Tamilnadu, India

ARTICLE INFO

Article History:
Received 19th January, 2012
Received in revised form
21th February, 2013
Accepted 11th March, 2013
Published online 13th April, 2013

Key words:
Amylase, *Aspergillus niger*,
Curcuma angustifolia,
Submerged fermentation,

ABSTRACT

Amylase was produced by *Aspergillus niger* utilizing *Curcuma angustifolia* as a carbon source in submerged fermentation. The effect of varying pH of the medium, temperature, carbon and nitrogen sources on the production of α- amylase was investigated. The maximum activity of α-amylase was recorded after 7 days of submerged fermentation at pH 5 and room temperature 28^{0}C. The enzyme produced by *Aspergillus niger* can be used in industrial process after characterization. The maximum amylase activity was recorded as 345 U/mg.

INTRODUCTION

Alpha-amylase is a hydrolytic enzyme and in recent years, interest in microbial production has increased dramatically due to its wide spread use in food, textile, baking and detergent industries (Asghar *et al*., 2000). α-amylase enzymes are important enzymes employed in starch processing industries for hydrolysis of polysaccharides such as starch into simple sugar constituents (Akpan *et al*., 1999; Fogarty and Kelly, 1980; Nigam and Singh, 1995). Although many microorganisms produce this enzyme, the most commonly used for their industrial application are *Bacillus licheniformis*, *Bacillus amylolycheniformis* and *Aspergillus niger*. Nowadays, the new potential of using microorganism as biotechnological source of industrially relevant enzymes has stimulated interest in exploration of extracellular enzymatic activities in several microorganisms (Akpan *et al*.,1999; Bilinski and Stewart, 1995; Buzzini and Martini, 2002). These enzymes are found in animals (Salva, Pancreas), plants (Malt), bacteria and molds (Abu *et al*., 2005).A growing new area of application of α-amylase is in the field of laundry and dish washing detergents (Van der Maarel *et al*., 2002).

Studies on fungal amylases especially *Aspergillus niger* have been concentrated because of their ubiquitous nature and non fastidious nutritional requirement (Abu *et al*., 2005). The production of α-amylases by molds has been greatly affected by cultural and nutritional requirement.Amylase of fungal origin was found to be more of stable than the bacterial enzymes on a commercial scale.Submerged fermentation holds tremendous potential for the production of enzymes. This method has economic value for countries with abundance of biomass and agro industrial residues, as these can be used as cheap raw materials (Tunga and Tunga, 2003). In the present work, the substrate *i.e. Curcuma angustifolia* is used for the production of α- amylase. Different process conditions were studied to achieve maximum yield of α- amylase production using various experimental designs.

***Corresponding author:** rajilachingans@gmail.com

MATERIALS AND METHODS

(I) Sample collection

Curcuma angustifolia (Commonly known as Arrow root) is obtained from the hilly areas of Malappuram and Cannanore districts of Kerala (India).

(II) Isolation of *Aspergillus niger*

Aspergillus species was isolated from the soil and maintained on potato dextrose agar (PDA).

Lactophenol Cotton Blue staining

A loop full of fungal cultures was placed on a clean glass slide, a drop of lactophenol cotton blue stain was mixed with the culture. A clean coverslip was placed over the culture and viewed under the microscope (45X) and the morphology of *Aspergillus niger* was observed and photographed.

(III) Sample preparation

The collected sample was washed thoroughly; the skins were removed and chopped into pieces which were then boiled. The boiled sample was grinded and the aqueous extract was obtained by squeezing it using muslin cloth. This extract was used as carbon source in the Czapek dox medium in which the *Aspergillus niger* was inoculated and kept for incubation for 7- days at 3 different temperatures(37^{0}C, 20^{0}C,and 28^{0}C) and 3 different PH (3.0, 5.O, and 7.0).

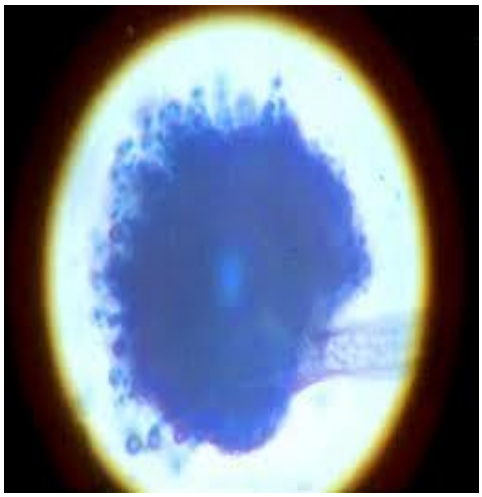

Plate 1. *A.niger* viewed under microscope after staining with LPCB

(IV) Extraction of Amylase from the Fermentation medium

After incubation, the fermentation medium was harvested by centrifugation at 10,000rpm for 10 minutes at 4^0C. The supernatant was collected and subjected for estimate the amylase activity.

Effect of temperature

To study the effect of temperature on amylase production, the submerged fermentation was carried out at different temperatures (20^0C, 37^0C, 28^0C).

Effect of pH

The fermentation medium was prepared by varying the p^H values (3.0, 5.0, and 7.0) for the production of amylase.

Effect of Nitrogen sources

The effect of nitrogen source on enzyme production was studied by replacing the nitrogen source in Czapek dox medium, p^H - 5.0 with organic nitrogen sources such as peptone, casein, yeast extract and inorganic nitrogen sources such as NH_4NO3, KNO3 and incubated at room temperature for 7 days.

Enzyme assay

Amylase activity was estimated by the analysis of reducing sugar released during hydrolysis of 1% (w/v) starch in 0.1 M sodium citrate buffer by the Dinitrosalicylic acid (DNS) method (Miller, 1959). One unit of amylase activity was defined as the amount of enzyme that releases 1mMol of reducing sugar as glucose per minute under assay condition.

Protein estimation

Protein content of the enzyme extracts was estimated by the method of Lowry. *et al*. (1951), using Bovine Serum Albumin as the standard.

RESULT AND DISCUSSION

Different fermentation parameters were optimized for α- amylase production by conducting series of experiments and the results are discussed as under.

Effect of temperature

Duplicate flask containing 30gm of fresh boiled tuber of *Curcuma angustifolia* was autoclaved, inoculated and incubated at different temperature of 37^0C, 20°C and room temperature (28°C) for seven days. The maximum activity of α- amylase was noted in the enzyme extracts incubated at room temperature of a pH-5 in *Curcuma angustifolia* (345 U/mg) (shown in Table-2 & Fig-2). It is reported that best enzyme production in *A.niger* at room temperature both in Submerged Fermentation and Solid State Fermentation (Varalakshmi et al., 2009) and reported 30°C be the best for enzyme production by *Penicillium fellutanum* (Kathiresan & Manivannan, 2006).

Effect of pH

Aspergillus niger was inoculated in *Curcuma angustifolia* was incubated at room temperature 28^0C for 7 days. The enzyme was extracted and the amylase produced at different pH was recorded. The maximum yield of amylase was in pH-7 and the amylase production was 345 U/mg in *C. angustifolia* (shown in Table 3 & Figure -3). In contrary to our results Varalakshmi et al,. (2009) reported the maximum enzyme activity of 75 U /mg of protein at pH- 9.5. Others have reported acidic pH optima for amylases from *A.niger* (Hernandes et al., 2006; Mitieri et al., 2006). Different organisms have different pH optima and decrease or increase in pH on either side of the optimum value results in poor microbial growth (Lehninger, 1982).

Effect of Nitrogen source

Addition of organic sources such as peptone, casein and yeast extract to the medium resulted in a considerable increase in the production of alpha amylase compared to the control (19.5U/mg in *Curcuma angustifolia*). Media supplemented with peptone showed maximum amylase activity compared to casein and yeast extract (Fig.4). Suganthi. R. *et al*. in the year 2011 reported that nitrogen source increases the yield of alpha amylase produced in ground nut oil cake medium. Similarly the supplementation with inorganic nitrogen sources to amylase production by *Aspergillus niger* is done with success increase in the yield of enzyme in submerged fermentation (Pandey, 2005). Among the inorganic nitrogen sources ammonium nitrate showed highest amylase activity (Fig.4). Our results are in agreement found that the nitrogen supplementation enhances the production of the organism and have increased in the biomass cropped (Anupama and Ravindra, 2001). Previous findings have shown that peptone, sodium nitrate and casein hydrolysate are good nitrogen supplements for amylase production in *A.fumigatus*,(Got *et al*.,1998) *A.niger* (Pandey *et al*.,1994) and *A.oryzae* (Pederson and Neilson,2000).

Tables and Figures indicating the effect of different parameters on Amylase production *Curcuma angustifolia*

Table 1. The effect of pH-3 at different temperature on fungal enzyme production after 7 days incubation

Concentration (µg)	p^H	Temperature	Amylase activity U/mg
50	3.0	20^0C	221.25
100	3.0	20^0C	150
50	3.0	28^0C	217.5
100	3.0	28^0C	322.5
50	3.0	37^0C	150
100	3.0	37^0C	206.25

Table 2. The effect of pH-5 at different temperature on fungal enzyme production after 7 days incubation

50	5.0	20^0C	150
100	5.0	20^0C	165
50	5.0	28^0C	225
100	5.0	28^0C	345
50	5.0	37^0C	187.5
100	5.0	37^0C	255

Table 3. The effect of pH-7 at different temperature on fungal enzyme production after 7 days incubation

50	7.0	20^0C	221.25
100	7.0	20^0C	165
50	7.0	28^0C	195
100	7.0	28^0C	288.75
50	7.0	37^0C	157.5
100	7.0	37^0C	228.75

Effect of Nitrogen sources on *Curcuma angustifolia*

Organic Nitrogen Sources

Nitrogen source	Concentration (µg)	pH	Temperature	Amylase activity(U/mg)
Inorganic Nitrogen sources				
NH_4NO3	50	5.0	28	125.75
NH_4NO3	100	5.0	28	375
KNO3	50	5.0	28	112.5
KNO3	100	5.0	28	352.5

Table 4. The effect of different nitrogen sources at temperature 28^{0}C, pH- 5 on fungal amylase production after 7 days incubation

Peptone	50	5.0	28	67.7
Peptone	100	5.0	28	82.5
Caesin	50	5.0	28	22.5
Caesin	100	5.0	28	67.7
Yeast extract	50	5.0	28	33.75
Yeast extract	100	5.0	28	56.25

Figures indicating the effect of different parameters on fungal enzyme production *Curcuma angustifolia*

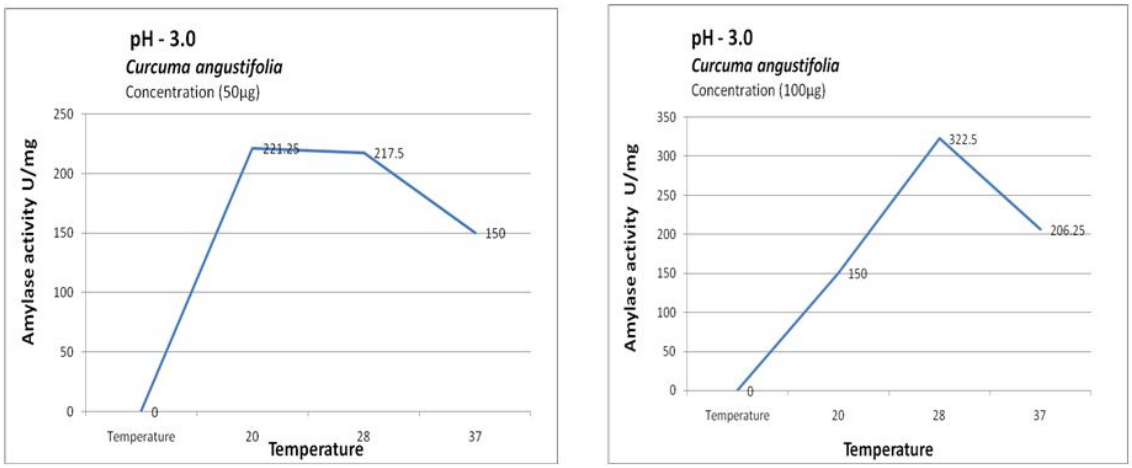

Fig. 1. The effect of pH-3 on amylase production at different temperatures

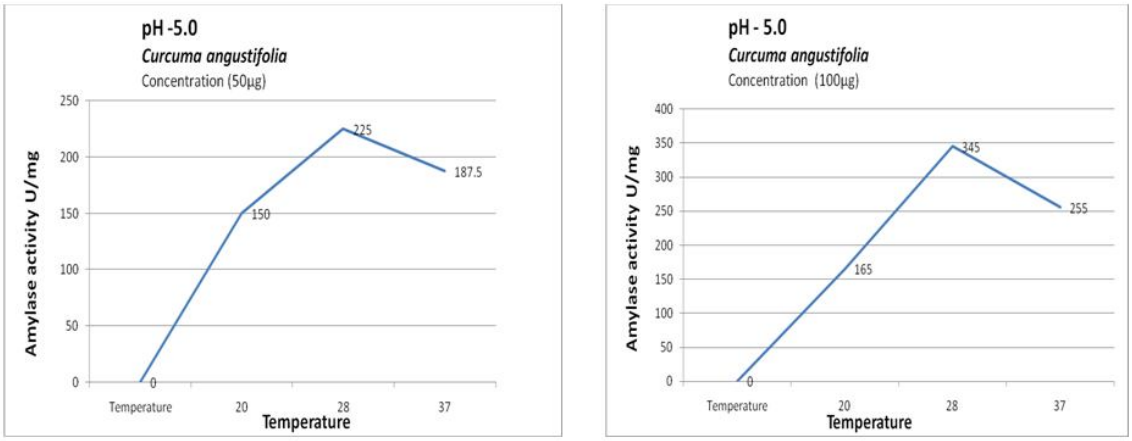

Fig. 2. The effect of pH-5 on amylase production at different temperatures

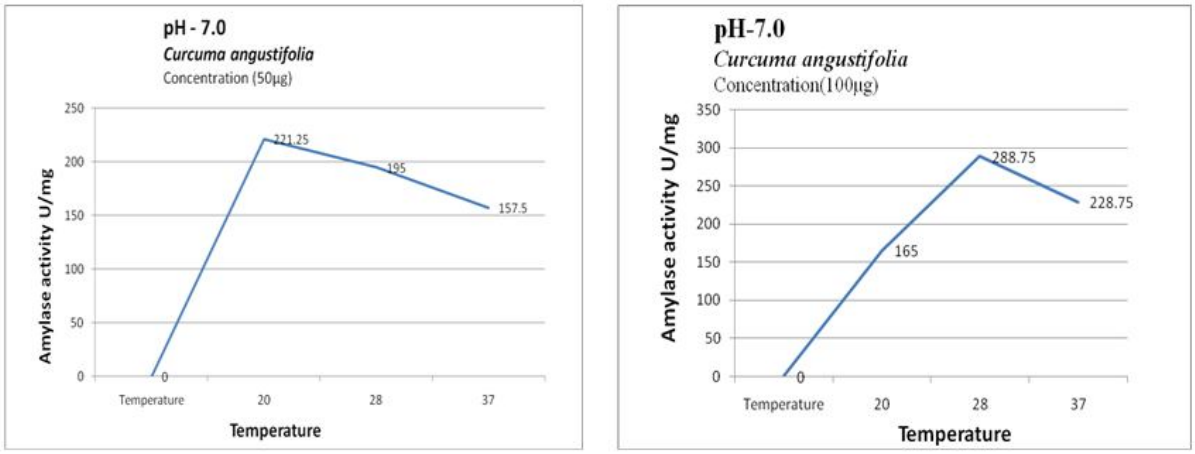

Fig. 3. The effect of pH-7 on amylase production at different temperatures

Figures indicating the effect of Nitrogen sources on *Curcuma angustifolia*

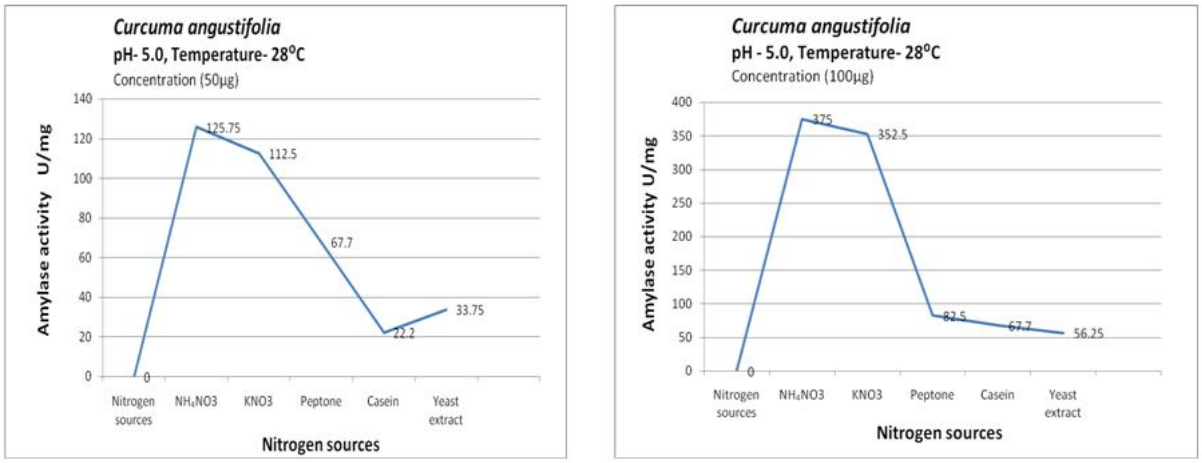

Fig. 4. Effect of different nitrogen sources on fungal amylase production at p^H-5.0 and temperature - 28°C

Conclusion

The maximum production of α- amylase was observed in tuber of *Curcuma angustifolia* at room temperature (28°C) with a pH of 5.0. Higher yield of α-amylase production from *A.niger* was possible by Submerged Fermentation. From the results the media supplemented with ammonium nitrate gave more biomass yields highest amylase activity. This highlights the importance of nitrogen sources to increase biomass yield. Apparently the preferred nitrogen source for amylase production by *A.niger* was ammonium nitrate. In conclusion the amylase activity was higher when *Curcuma angustifolia* was used as substrate at a p^H 5.0 in 28°C.

Acknowledgement

The authors thank to Ms. P. Suganyadevi for her keen interest and encouragement. The authors would also like to thank Mrs. Kavitha Krishna, Dr. Saravanakumar and Dr. M. Aravindhan for valuable discussion and suggestions.

REFERENCES

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

Akpan I, Bankjole Mo, Adesermowo Am and Lantunde – Data 1999. Production of α- amylase by *Aspergillus niger* in a cheap solid medium using rice bran and agricultural material. *Trop. Sci*; 39; 77-79.*Braz. Arc. Biol. Tech* 2001; 44: 79-88.

Anupama and Ravindra P, Studies on production of single cell protein by A.niger in solid state fermentation of rice bran.

Asghar, M, M.J. Asad and M. Arshad, 2000. Alpha- amylase from *Aspergillus niger* in waste bread medium. Proc.2nd Int.Symp, Technol. For Environ. Monit and Agro- Appil. October 18-20, Tekirdag, Turkey.

Bilinski and Stewart.1995. Production and Characterization of α-amylase from *Aspergillus niger*.18: 551-556.

Buzuhi and Martini. 2002. Extracellular enzymatic activity profiles in yeast and yeast like strains isolated from tropical environments. *Journal of Applied Microbiology*. 93: 1020-1025.

Fogarty WM and Kelley C T. 1980.Amylase, amyloglucosidase and related glucanases. *In; Microbial Enzymes and bioconversions*, Rose, A.H (Ed). Academic Press, London. 115-170.

Hernandez MS, Marilu RR, Nelson PG and Renato PR.2006. Amylase production by *Aspergillus niger* in submerged cultivation on two wastes from food industries. *J. Food Eng*. 73: 93-100.

Kathiresan K and Manivannan S. 2006.Amylase production by *Penicillium fellutanum* isolated from Mangrove rhizosphere soil.

Lowry O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall. 1951. Protein measurement with the Folin- Phenol reagents. *J. Biol. Chem*. 48: 17-25.

Miller G.L. 1959. Use of Dinitrosalicylic acid reagent for determination of reducing sugar, *Anal*. Chem. 31, 426-429.

Nigam.P, and Singh D.1995. Enzymes and microbial enzymes involved in starch processing enzymes *Microb. Technol*.17: 770-778.

Pandey A, Selvakumar P and Ashakumary L.1994. Glucose production by *A.niger* on rice bran is improved by addition of nitrogen sources.*World J.Microbial. Biotechnol.* 10:348-349.

Pandey A, Webb C, Soccol CR,LArroche C.2005. Rice bran as a substrate for proteolytic enzyme production. *Enzyme technology*, New Delhi: Asiatech Publishers, Inc.197.

Pederson H and Neilson J.2000. The influence of nitrogen sources on alpha amylases productivity of *A.oryzae* in continuous cultures. *Appl.Microbiol.Biotechnol*. 53:278-281.

Tunga R and Tunga B S.2003. Extracellular amylase production by *Aspergillus orizae* under solid state fermentation. International Centre for Biochemistry, Olaka University, Japan. 12.

Van der Maarel, M.J.E.C., B.Van der Veen, J.C.M. Vitdehaag, H. Leemhuis and L. Dijkhuizen. 2002. Properties and application of starch converting enzymes of α-amylase family.*J. Biotechnol.* 49: 137- 55.

Varalakshmi KN, Kumudini BS, Nandini BN, Solomon J, Suhas R, Mahesh B and Kavitha AP.2009.Production and characterization of alpha amylase from *Aspergillus niger* JGI 24 isolated in Bangalore. *Polish Journal of Microbiology*. 58: 29-36

****