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EFFECT OF CULTIVATION CONDITIONS ON ACTIVITY OF α-AMYLASE FROM A TROPICAL STRAIN *ASPERGILLUS FLAVUS* LINK

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ABSTRACT

An important integral part of the physiology of the fungal cell is the nature of the cascade of extracellular enzymes produced by fungi. A tropical strain *Aspergillus flavus* Link obtained from deteriorated tomato (*Solanum lycopersicum*) fruits grew in a growth nutrient medium composed of $MgSO_4.7H_20$, K_2HPO_4, KH_2PO_4, $FeSO_4.7H_2O$, potassium nitrate and starch at 30°C. Extracellular proteins produced by the isolate in the medium expressed α-amylase activity. The enzyme was partially purified by ammonium sulphate precipitation followed by dialysis. The enzyme exhibited optimum activity at 35°C and at pH 6.0. It possessed an apparent K_m of 7.1 mg/ml for the hydrolysis of starch. The enzyme was stimulated by Na^+ and Ca^{2+} but inhibited by ethylenediamine tetraacetic acid and 2,4-dinitrophenol. It lost 64.1% of its activity within 20 min of heating at 80°C. Of the nitrogenous compounds potassium nitrate, ammonium sulphate and casein, casein as nitrogen source in the defined growth medium with starch as carbon source supported most activity of α-amylase by the fungal isolate. Observations during the partial purification and characterization of the enzyme are herein reported.

Keywords: α-Amylase, *Aspergillus flavus* Link, tropical strain, tomato

INTRODUCTION

Amylases are hydrolytic enzymes that catalyze the degradation of starch molecules and other carbohydrates to yield dextrins and progressively smaller polymers composed of glucose units (**Bohinski, 1983; Reddy *et al.*, 2003**). They can be of animal, plant or microbial sources (**Wu *et al.*, 2004; Rahardjo *et al.*, 2005**). The two types of amylases commonly encountered in microbial degradation of starch are α- and β-amylases. Investigations on two hundred and ninety-six bacterial isolates for the effects of saliva and α-amylase on their susceptibility to ampicillin, tetracycline, chloramphenicol and gentamicin have shown that α-amylase significantly reduced the Minimum Inhibitory Concentration of tetracycline from 2.0 to 0.25 mg/ml. However, with *Staphylococcus aureus*, priming with saliva and α-amylase had no effect on the Minimum Inhibitory Concentrations of gentamicin and ampicillin, whereas, the Minimum Inhibitory Concentrations of tetracycline and chloramphenicol were increased (**Eke, 1984**). According to **Siegel *et al*.** (**1999**), Sodium orthovanadate used at concentration 3 x 10^{-3}M stimulated amylase release by the pancreas by 40%. Two organophosphates: endosulphan and methyl parathion stimulated amylase activity in the midgut of the larva of the fruit-sucking moth, *Orthreis maternal* L. (*Lepidoptera*: Nocturidae) (**Deshmukh and Tembhare, 1998**). **Wang *et al.*** (**1998**) reported changes in secretory amylase activity in Wistar strain rats fed on low calcium diet. Proteinuria and hematuria were related to high urinary amylase level in some individuals with alcohol dependence syndrome (**Ito *et al.*, 1999**). According to **Tsuchida *et al.*** (**1999**), adrenomedullin inhibits calcium induced amylase secretion by reducing calcium sensitivity of the exocytosis machinery of the pancreatic acini. Amylase is a key enzyme involved in digestion and carbohydrate metabolism in silkworm and its activity is related to productivity traits. It is found in the digestive juices of new bivoltine races of *Bombyx mori* (**Maribashetty *et al.*, 1998**). According to **Lavon and Goldschmidt** (**1999**), amylase activity inversely correlated citrus leaf K levels. A decreased metabolism of serum amylase in patients with chronic infective liver disease especially in those having liver cirrhosis, may lead to accumulation of this enzyme in the blood (**Raffaele *et al.*, 1999**). Studies carried out on post-mortem keeling yam tubers show that starch degradation enzyme, amylase, fluctuated during storage (**Chu *et al.*, 1998**). According to **Hristov *et al.*** (**1998**), amylase was resistant to pepsin but irreversibly inactivated at low pH. Studies on a synthetic pyrethroid insecticide, Cypermethrin, administered as formulation, Ripcord 25EG (emulsified concentrate), administered to adult beetles of a stored grain pest, *Tribolium castaneum* revealed amylase sensitivity to sub-lethal doses of the insecticide. The effects were dependent on duration of treatment (**Saleem *et al.*, 1998**). **Zhuravlev *et al.*** (**1998**) reported elevated content of amylase in pleural exudates of tumor nature. Amylase activity increased by 84% after fifteen days of DDT administration to Sprague Dawley rats at three different doses of 100, 20, and 10 mg/Kg body weight (**Shahid and Shakoon, 1999**). Increased total serum amylase activity and pancreatic amylase activity were more frequent in patients with diabetic ketoacidosis than in patients with poorly controlled non-ketotic diabetes and in patients with well-controlled diabetes (**Vantyghem *et al.*, 1999**). Studies carried out on the metabolic changes in insects reared on artificial diet containing royal jelly show that the presence of royal jelly in substrate does not significantly affect the specific activity of the midgut amylase (**Nenadovic *et al.*, 1999**). Alpha amylase inhibits growth of *Neisseria gonorrhoeae*, *Legionella pneumophilia* and also of *Neisseria meningitidis* (**Berger, 1984**). Among bacterioneusten and bacterioplankton from the Baltic Sea, the region of Gdansk Deep, amylolytic organisms were less numerous (about 5%) physiologic group with no differences in activity level between bacterial amylases from various water levels (**Mudryk, 1998**). *Saccharomycopsis fibuligera*, *Saccharomycopsis capsularis* and *Pichia burtoni* isolated from marcha (used to produce alcoholic drinks in India, Nepal, Bhutan and Tibet in China) had high amylolytic activities (**Tsuyoshi *et al.*, 2005**).

Aspergillus flavus Link is associated with post-harvest rot of tomato (*Solanum lycopersicum*) fruits in the tropics. *Aspergillus flavus* isolated from soil sample in Gwagwalada, Federal Capital Territory, Abuja, Nigeria were reported as capable of production of amylase (**Ugoh and Ijigbade, 2013**). According to **Saleem and Ebrahim** (**2014**), *Aspergillus flavus* isolated from seed samples of legumes in Almadinah Almunawwarah Saudi Arabia was capable of production of α-amylase at 28°C. **Ali *et al.*** (**2017**) reported that *Aspergillus flavus* produced α-amylase in submerged fermentation using mandarin peel within 4 – 5 days at 28 – 40°C and at pH 4 – 5.5.

Tomato is lost to post-harvest pathogens both in the tropics and temperate regions of the world. This work is aimed to study the effect of cultivation conditions such as temperature, pH, and composition of nutrient growth medium such as substrate concentrations, certain cations and specific inhibitors on increase of activity of

extracellular α-amylase produced by tropical fungal strain *Aspergillus flavus* isolated from deteriorated tomato (*Solanum lycopersicum*) fruits. This is with the view to prolonging the shelf-life of the fruits lost to this post-harvest pathogen.

MATERIALS AND METHODS

Source and Identification of Isolate

The tropical fungal strain *Aspergillus flavus* Link for this investigation was isolated from deteriorated tomato (*Solanum lycopersicum*) fruit obtained from Cocoa Research Institute, Ibadan, Nigeria (CRIN). The fungal isolate was identified at the Seed Health Unit of the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. The isolate was cultured on potato dextrose agar (Lab M) on slants and plates.

Culture conditions and inoculum

The tropical fungal strain *Aspergillus flavus* Link isolated from deteriorated tomato (*Solanum lycopersicum*) fruit was grown in a defined growth medium with specific nitrogen and carbon sources of fungal growth. The isolate was cultured and maintained on potato dextrose agar slants and plates. The fungus was sub-cultured on the test tubes of the same medium and incubated at 30^{o}C. The ninety six-hour-old culture of isolate was used for this study. Based on a modified method of **Adejuwon and Tsygankova** (**2017**), culture was grown in a defined growth medium composed of $MgSO_4.7H_20$ (0.1 g), K_2HPO_4 (2 g), KH_2PO_4 (0.5 g), $FeSO_4.7H_2O$ (1 mg), KNO_3 (9.9 g) and starch (10 g) source (Sigma-Aldrich, USA) per 1 litre of distilled water. Conical flasks (250 ml) containing 50 ml growth medium were inoculated with 1 ml of an aqueous spore suspension containing approximately 6 x 10^5 spores per 1 ml of isolate. Experimental and control flasks were incubated without shaking at 30^0C (**Olutiola and Nwaogwugwu, 1982**). Protein content of the inoculated medium was determined using the **Lowry *et al.*** (**1951**) method.

Extraction of Enzyme

On the tenth day of inoculation of growth medium, the contents of flask were carefully filtered through glass fibre filter paper (Whatman GF/A). The protein content of the filtrate was determined (**Lowry *et al*., 1951**). The filtrate was also assayed for α-amylase activity (**Pfueller and Elliott, 1969**).

Ammonium Sulphate Fractionation

The crude enzyme was treated with ammonium sulphate (analytical grade, Sigma) at 90% saturation (662 g/L). Precipitation was allowed at 4^{o}C for 24 h. The mixture was centrifuged at 4,000 rpm for 30 minutes at 4^{o}C using a high speed cold centrifuge (Optima LE-80K Ultracentrifuge, Beckman, USA). The supernatant was discarded. The precipitate was re-constituted in 0.2 M citrate phosphate buffer (pH 6.0). The protein content of the precipitated enzyme was determined (**Lowry *et al.*, 1951**). α-Amylase activity was also determined (**Pfueller and Elliott, 1969**).

Dialysis

The ammonium sulphate precipitated enzyme was dialyzed using a visking dialysis tubing (**Whitaker *et al.* 1963**). The enzyme preparation was dialyzed using 0.2 M citrate phosphate buffer (pH 6.0) at 4^{o}C for 18 h. The protein content of the dialyzed enzyme was afterwards determined (**Lowry *et al.*, 1951**). α-Amylase activity of the dialyzed enzyme was also determined (**Pfueller and Elliott, 1969**).

Enzyme Assay

α-Amylase

α-Amylase activity was monitored using the method of **Pfueller and Elliott** (**1969**). Reaction mixture consisted of 2 ml of 0.1% (w/v) starch in 0.2 M citrate phosphate buffer (pH 6.0) as substrate and 0.5 ml enzyme. Controls consisted of 2 ml of substrate. The contents of both experimental and control tubes were incubated at 35^{o}C for 1 hr. The reaction in each tube was terminated with 3 ml of 1 N HCl afterwards; 0.5 ml enzyme was added to the control tubes. The 2ml mixture of each experimental and control was transferred to new sets of test tubes. The 3ml of 0.1 N HCl was added into the contents of each test tube after which 0.1 ml of iodine solution was added. Optical density readings were taken at 620nm. One unit of α-amylase activity was defined as the amount of α-amylase which produced 1 percent reduction in the intensity of the blue colour of starch-iodine complex under assay conditions. Specific activity was calculated and expressed as enzyme units per mg protein.

Characterization of the Partially Purified α-Amylase

The effects of temperature, pH, substrate concentrations, certain cations and specific inhibitors on the activity of the partially purified α-amylase from *Aspergillus flavus* Link were investigated.

Effect of temperature

The substrate used was 0.1% (w/v) starch dissolved in 0.2 M citrate phosphate buffer, pH 6.0. The reaction mixture was 2 ml of substrate and 0.5 ml of enzyme. Incubation was performed at a range of 4-60^{o}C for 1 hr.

Stability test at 80^{o}C

The effect of heat (80^{o}C) on the stability of the partially purified enzyme at different periods, 2, 5, 10, 15 and 20 minutes was examined. The activity of the heated enzyme was determined by incubating 0.5 ml of enzyme with 2 ml of the citrate phosphate buffer (pH 6.0), and 0.1% starch substrate at 35^{o}C for 1 hr.

Effect of pH

The substrate used was 0.1% (w/v) starch dissolved in 0.2 M citrate phosphate buffer at different pH values ranging from pH 3.0 – 8.0. The reaction mixture was 2 ml of substrate and 0.5 ml of enzyme. Incubation was performed at 35^{o}C for 1 hr.

Effect of substrate concentrations

Different concentrations, 0.05 – 0.3% (w/v) of starch (Sigma-Aldrich, USA) dissolved in 0.2 M citrate phosphate buffer, pH 6.0 were used as substrate. The reaction mixture was 2 ml of substrate and 0.5 ml of enzyme, incubated at 35^{o}C for 1 hr.

Effects of certain cations

The effects of cations of NaCl and $CaCl_2$ used at various concentrations (5 x 10^6, 10 x 10^6, 15 x 10^6, 20 x 10^6 and 30 x 10^6 nM) on the activity of the partially purified α-amylase were examined. Each salt was added in 0.1% starch dissolved in citrate phosphate buffer at pH 6.0. The reaction mixture consisting from 2 ml of substrate and 0.5 ml of enzyme was incubated at 35^{o}C for 1 hr.

Effects of specific inhibitors

The 2,4-dinitrophenol and ethylenediamine tetraacetic acid used at various concentrations (2 x 10^6, 4 x 10^6, 6 x 10^6, 8 x 10^6 and 10 x 10^6 nM) were added in 0.1% starch (Sigma-Aldrich, USA) dissolved in citrate phosphate buffer at pH 6.0. These were used as substrates.

Effects of different nitrogenous compounds

The effects of different nitrogenous compounds (potassium nitrate, ammonium sulphate and casein) used as nitrogen source, and starch used as carbon source in the defined growth medium on α-amylase activity expressed by the tropical fungal strain *Aspergilllus flavus* Link were investigated. α-Amylase activity expressed on the tenth day of incubation at 30^{o}C was recorded.

RESULTS

The tropical fungal strain *Aspergilllus flavus* Link (Figure 1) isolated from deteriorated tomato (*Solanum lycopersicum*) fruits grew in a defined medium composed of $MgSO_4.7H_20$, K_2HPO_4, KH_2PO_4, $FeSO_4.7H_2O$, potassium nitrate and starch at 30^{o}C.

Figure 1 The tropical fungal strain *Aspergilllus flavus* Link isolated from deteriorated tomato (*Solanum lycopersicum*) fruits cultured on potato dextrose agar in plates at 30^{o}C

Extracellular proteins produced within ten days of incubation at this temperature expressed α-amylase activity. The crude enzyme was partially purified by ammonium sulphate precipitation followed by dialysis. The purification steps are represented in Table 1. Enzyme yield after ammonium sulphate fractionation followed by dialysis was 22.14%. Purification fold was 4.2 (Table 1).

Table 1 Partial purification of α-amylase obtained from tropical strain *Aspergillus flavus* Link

Fraction	Total activity (U)	Total protein (mg)	Specific activity (U/mg protein)	Yield (%)	Purification fold
Crude enzyme	1418	11.4	124.4	100 22.14	1 4.2
Ammonium sulphate precipitation (90% saturation)	314	0.6	523.3		

The partially purified α-amylase was characterized. Within a temperature range of 4 - 60ºC, optimum α-amylase activity was observed at 35ºC and was equal to 539 ± 0.01 Units per mg protein (Table 2).

Table 2 Effect of temperature on the activity of partially purified α-amylase obtained from tropical strain *Aspergillus flavus* Link

Temperature (ºC)	α-Amylase activity (U/mg protein)
4	3.4 ± 0.03
20	320 ± 0.01
30	458 ± 0.02
35	539 ± 0.01
50	423 ± 0.02
60	412 ± 0.03

*Mean values with standard errors calculated from data of triplicates

Within a pH range of 3.0 - 8.0, optimum α-amylase activity was observed at pH 6.0 and was equal to 546 ± 0.01 Units per mg protein (Table 3).

Table 3 Effect of pH on the activity of partially purified α-amylase obtained from tropical strain *Aspergillus flavus* Link

pH	α-Amylase activity (U/mg protein)
3.0	82 ± 0.01
4.0	426 ± 0.01
5.0	523 ± 0.02
6.0	546 ± 0.01
7.0	416 ± 0.02
8.0	210 ± 0.01

*Mean values with standard errors calculated from data of triplicates

Using a starch concentration range of 0.5 mg/ml – 3 mg/ml as substrate, optimum α-amylase activity was observed at 2 mg/ml and 3 mg/ml and was equal to 554 ± 0.01 Units per mg protein (Table 4).

Table 4 Effect of substrate concentration on the activity of partially purified α-amylase obtained from tropical strain *Aspergillus flavus* Link

Starch concentration (mg/ml)	α-Amylase activity (U/mg protein)
0.5	166 ± 0.02
1	255 ± 0.03
2	554 ± 0.01
3	554 ± 0.01

*Mean values with standard errors calculated from data of triplicates

The enzyme possessed an apparent Km of 7.142 mg/ml for the hydrolysis of starch (Figure 2).

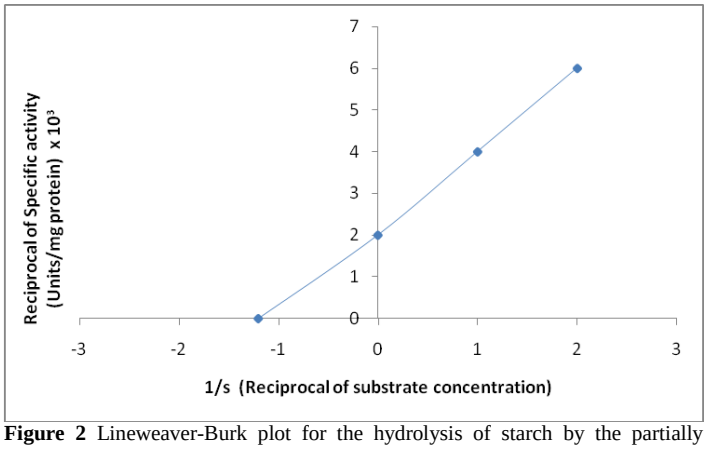

Figure 2 Lineweaver-Burk plot for the hydrolysis of starch by the partially purified α-amylase obtained from tropical strain *Aspergillus flavus* Link

The activity of the partially purified α-amylase produced by *Aspergillus flavus* Link was stimulated by increasing concentrations of Na^+ and Ca^{2+} used. The used concentrations were from 5 x 10^6 to 30 x 10^6 nM. There was a steady increase in α-amylase activity with increasing cation concentrations within these ranges. α-Amylase activity was equal to 573 ± 0.02 Units per mg protein when using Na^+ at concentration of 5 x 10^6 nM. Activity steadily increased with increasing concentration of the cation and was equal to 628 ± 0.03 Units per mg protein at 30 x 10^6 nM concentration of Na^+ (Table 5).

Table 5 Effect of Na^+ on the activity of partially purified α-amylase obtained from tropical strain *Aspergillus flavus* Link

Concentration of cation (nM)	α-Amylase activity (U/mg protein)
5 x 10^6	573 ± 0.02
10 x 10^6	582 ± 0.01
15 x 10^6	593 ± 0.01
20 x 10^6	612 ± 002
30 x 10^6	628 ± 0.01

*Mean values with standard errors calculated from data of triplicates

Also, α-amylase activity was equal to 598 ± 0.01 Units per mg protein when using of Ca^{2+} at concentration 5 x 10^6 nM. Activity increased steadily with increasing concentration of the cation and was 950 ± 0.02 Units per mg protein at 30 x 10^6 nM concentration of Ca^{2+} (Table 6).

Table 6 Effect of Ca^{2+} on the activity of partially purified α-amylase obtained from tropical strain *Aspergillus flavus* Link

Concentration of cation (nM)	α-Amylase activity (U/mg protein)
5 x 10^6	598 ± 0.02
10 x 10^6	791 ± 0.03
15 x 10^6	854 ± 0.01
20 x 10^6	876 ± 001
30 x 10^6	950 ± 0.02

*Mean values with standard errors calculated from data of triplicates

The activity of the partially purified α-amylase from the tropical *Aspergillus flavus* Link was inhibited by ethylenediamine tetraacetic acid and 2, 4-dinitrophenol used at concentrations ranging from 2 x 10^6 nM to 10 x 10^6 nM, respectively. The activity of the partially purified α-amylase obtained from the fungus was equal to 514 ± 0.02 Units per mg protein when using ethylenediamine tetraacetic acid at concentration 2 x 10^6 nM, and activity of α-amylase had decreased to 338 ± 0.01 Units per mg protein at concentration of this inhibitor 10 x 10^6 nM (Table 7).

Table 7 Effect of ethylenediamine tetraacetic acid on the activity of partially purified α-amylase obtained from tropical strain *Aspergillus flavus* Link

Concentration of cation (nM)	α-Amylase activity (U/mg protein)
2 x 10^6	514 ± 0.02
4 x 10^6	502 ± 0.01
6 x 10^6	449 ± 0.01
8 x 10^6	393 ± 002
10 x 10^6	338 ± 0.01

*Mean values with standard errors calculated from data of triplicates

Also, the activity of the partially purified α-amylase from the fungus was equal to 422 ± 0.02 Units per mg protein when using 2,4-dinitrophenol at concentration 2 x 10^6 nM, and activity of α-amylase had decreased to 103 Units per mg protein at concentration of this inhibitor 10 x 10^6 nM (Table 8).

Table 8 Effect of 2, 4-dinitrophenol on the activity of partially purified α-amylase obtained from tropical strain *Aspergillus flavus* Link

Concentration of cation (nM)	α-Amylase activity (U/mg protein)
2 x 10^6	422 ± 0.02
4 x 10^6	386 ± 0.02
6 x 10^6	308 ± 0.03
8 x 10^6	279 ± 002
10 x 10^6	103 ± 0.01

*Mean values with standard errors calculated from data of triplicates

The partially purified α-amylase from *Aspergillus flavus* Link was affected by heat. Significantly, the enzyme had lost 64.1% of its activity within 20 min of heating at 80°C (Table 9).

Table 9 Effect of heat stability test at 80°C on the activity of partially purified α-amylase obtained from tropical strain *Aspergillus flavus* Link

Time (minutes)	α-Amylase activity (U/mg protein)
2	398 ± 0.01
5	380 ± 0.02
10	365 ± 0.01
15	279 ± 003
20	143 ± 0.02

*Mean values with standard errors calculated from data of triplicates

Potassium nitrate, ammonium sulphate and casein used as nitrogen sources of fungal growth independently supported α-amylase production by the tropical strain *Aspergillus flavus* Link. The nitrogenous compounds used in this investigation as nitrogen source in accomplishment with starch as carbon source and casein supported α-amylase activity equal to 327 ± 0.02 Units per mg protein on the tenth day of incubation at 30°C (Table 10).

Table 10 Activity of α-amylase from *Aspergillus flavus* Link in defined growth with different nitrogenous compounds

Nitrogen source in substrate	α-Amylase activity (U/mg protein)
Potassium nitrate	129 ± 0.02
Ammonium sulphate	10.8 ± 0.03
Casein	327 ± 0.02

*Mean values with standard errors calculated from data of triplicates

DISCUSSION

The tropical isolate *Aspergillus flavus* Link obtained from deteriorated tomato (*Solanum lycopersicum*) fruits produced α-amylase in a defined growth medium with starch used as carbon source and with potassium nitrate, ammonium sulphate or casein used as nitrogen source for growth and metabolism. According to **Legin *et al.*** (**1997**), *Thermococcus hydrothermalis* and *Thermococcus fumicolans* isolated from deep sea were amylase producers. According to **Adejuwon *et al.*** (**2015**) from a previous investigation, a tropical strain of *Aspergillus flavus* isolated from bread was capable of production of α-amylase with either potassium nitrate or ammonium suphate as nitrogen source in their growth medium.

In this investigation, the partially purified α-amylase from the tropical strain *Aspergillus flavus* Link expressed optimum activity at 35°C and at pH 6.0. An extracellular α-amylase isolated from cell free broth of *Streptomyces megasporus* grown in glucose, soluble starch and raw starch was stable at a pH ranging from 5.5 to 8.5 but with optimum activity at pH 6.0 (**Dey and Agarwal, 1999**). Alpha amylase from *Thermoactinomyces vulgaris* had optimum activity at pH 4.8 to 6.0 (**Heese *et al.*, 1991**). Lactic acid was found to be produced from *Lactobacillus delbrueckii* subsp. delbrueckii and defatted rice bran powder containing starch with coupled saccharification with amylase at 37°C and pH 5.0 (**Tanaka *et al.*, 2005**). Studies by **Yuen *et al.*(1998)** on wood decay fungi (*Ascomycetes* and *Hyphomycetes*) isolated from submerged wood collected in tropic and subtropic fresh water revealed optimum temperatures of 20°C and 25°C for growth and amylase production.

The cations of NaCl and $CaCl_2$ stimulated activity of the partially purified α-amylase produced by the tropical strain *Aspergillus fluvus* Link in this investigation. According to **Mijts and Patel** (**2002**), the thermophilic, moderately halophilic anaerobic *Halothermothrix orenii* was able to synthesize α-amylase active with specific activity of 2,232 Units per mg protein requiring $CaCl_2$ for optimum activity and thermostability. A salt-tolerant thermostable amylase produced by *Bacillus megaterium* was reported to be stable at 5M NaCl (**Jana *et al.*, 1997**). A thermophilic *Thermoascus aurantiacus* has been observed to produce amylase with thermostability enhanced by calcium chloride (**Ohno *et al.*, 1998**). Amylase production from *Bacillus sphaericus* was reported to be maximum with 3mM divalent cations Mg^{++} and Ca^{++} incorporated in a growth medium (**Shekhar *et al.*, 1997**). Cadmium, Cobalt, Copper, Manganese, Nickel and Lead incorporated into Czapek-Dox liquid medium supported growth and production of amylase by soil yeasts *Geotrichum capitatum* and *Geotrichum candidum* (**Falih, 1998**). Activity of α-amylase from a marine *Vibrio sp* was found to be restored by Fe^{2+}, Mn^{2+}, Co^{2+}, Ca^{2+}, Mg^{2+} and Cu^{2+} to nearly 25-55% (**Najafi and Kembhavi, 2005**). A *Bacillus sp* produced an alkaliphilic amylase which was enhanced by Na^+ and Co^{2+} (**Bernhardsdotter *et al.*, 2005**).

The stimulatory actions of the cations Na^+ and Ca^{2+} is suggestive of a metal-requiring fungal α-amylase.

Activity of α-amylase from the tropical strain *Aspergillus flavus* Link was inhibited by ethylenediamine tetraacetic acid and 2,4-dinitrophenol in this research study. Amylase from *Fusarium verticillioides* has been observed to be inhibited by a hydrophobic 19.7-KDa inhibitor from corn kernel (**Figueira *et al.*, 2002a**). *Fusarium moniliforme*, a mycotoxigenic fungus has been reported to produce an amylase inhibited by a specific amylase inhibitor found in corn (**Figueira *et al.*, 2002b**). A strain of *Streptomyces nigrifaciens* has been reported to produce an amylase inhibitor having inhibitory effects on α-amylase and glucoamylase (**Su *et al.*, 1984**). A 14-kDa trypsin inhibitor associated with resistance to *Aspergillus flavus* and aflatoxin in maize also inhibited the α--amylase from *Aspergillus flavus*, indicating its bifunctional inhibitory function (**Fakhoury and Woloshuk, 1999**).

The inhibitory actions of ethylenediamine tetraacetic acid, a chelating agent, is indicative of a metalloenzyme nature of this fungal α-amylase. Also, the inhibitory actions of 2,4-dinitrophenol (an un-coupler of oxidative phosphorylation) further indicates metabolic energy playing a vital role in the actions of this fungal α-amylase.

The findings of this investigation may relate to the production of the enzyme α-amylase by the tropical strain *Aspergillus flavus* Link as a possible cause of degradation of the carbohydrate component of tomato (*Solanum lycopersicum*) fruits at postharvest. Starch synthesis in tomato fruits remains constant throughout fruit development (**Wang *et al.*, 1993; N'tchobo *et al.*, 1999**). Carbohydrates make up 4% of raw tomatoes, which amounts to less than 5 grams of carbohydrate for an average sized tomato of 123 grams. Simple sugars such as glucose and fructose make up almost 70% of this carbohydrate (**Bjarnadottir, 2015**). The physiological significance of α-amylase in deteriorating tomato fruits may be the hydrolysis of the carbohydrate portion of the tomato fruits.

CONCLUSION

The growth nutrient medium composed of $MgSO_4.7H_20$, K_2HPO_4, KH_2PO_4, $FeSO_4.7H_2O$ without the vitamins thiamine and biotin but with casein, ammonium sulphate or potassium nitrate used as nitrogenous growth source and starch used as carbon source will support the production of α-amylase by *Aspergillus flavus* Link at 30°C. Conditions inhibiting this enzyme will aid in prolonging the shelf-life of tomato fruits lost to this post-harvest pathogen both in the tropics and temperate zones of the world.

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