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Effect of Cations and Chemicals on the Activity of Partially Purified Cellulase from Tomato (*Lycopersicon esculentum* Mill) Fruits Deteriorated by *Aspergillus flavus* Linn

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Abstract: Within ten days of incubation, freshly ripe tomato fruits (Ibadan local variety) obtained from a local market in Ile-Ife, Osun State, Nigeria had completely deteriorated and proteins which possessed cellulolytic activity were released. The enzyme was partially purified by a combination of ammonium sulphate precipitation, molecular exclusion chromatography and ion-exchange chromatography. The enzyme was stimulated to varying degrees by Ca^{++}, Mg^{++}, Na^{+} and K^{+} but was inhibited by ethylene diamine tetraacetic acid, 2, 4-dinitrophenol and mercuric chloride.

Key words: Tomato fruits, cellulolytic activity, ion-exchange, chromatography, *Aspergillus flavus*

Introduction

The Ibadan local variety of tomato fruits was used for this research work. It is a local variety commonly found on farmers fields in South west Nigeria. The plant has indeterminate fruiting habit and it is highly straggling in growth habit. It is naturally high yielding and the fruits are conspicuously ridged and highly susceptible to cracking. Diseases caused by infection from microorganism on tomato fruits remains a serious problem of tomatoes(Khan *et al*., 1997; Mertelik and Mokra, 1998). The correct environmental conditions under which tomato fruits should be stored to minimize or completely eradicate the rot of tomato fruits in Nigeria is unknown to the market women who deal directly with these fruits from the farms.

Enzymes are so important in the degradation of the host tissues as this obviously affects their virulence (Famurewa and Olutiola, 1991). The production of enzymes in host-pathogen reactions cannot be over-emphasized. Cellulases are enzymes believed to play important roles in these degradation processes (Sethuraman *et al*., 1998) Cellulase have been reported to be associated with the pathogenicity of a number of microorganisms (Kalogeris *et al*., 2003, Jan and Chen, 2003). Cellulase was present in a mixture which enhanced the feed digestibility and milk production in four lactating annulated Holstcin cows (Beauchemin *et al*., 1999). The experiments described in this paper were designed to investigate the effects of some chemicals and cations on the activity of the partially purified cellulase from tomato fruits deteriorated by *Aspergillus flavus* Linn.

Materials and Methods

The isolate of *Aspergillus flavus* employed for this work was obtained from the culture collection of professor P.O Olutiola of the Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria. The organism was earlier on isolated from tomato fruit deteriorated in and was routinely grown and maintained on 1% (w/v) sabouraud dextrose agar slants. The organism was subcultured on sabouraud dextrose agar plates and incubated at 27°C. Seventy two-hour-old culture of the organism was used in inoculating healthy tomato fruits obtained from the Ile-Ife main market. Each fruit was inoculated with 4mm disc obtained from the sabouraud dextrose agar plate culture. Control fruits were similarly inoculated but with sterile sabouraud dextrose agar discs. Inoculated fruits were incubated under bell jars at room temperature of 27°C.

After incubation for ten days, the fruits were chilled and homogenized with cooled liquid extractant. The liquid extractant was 0.5M NaCl dissolved in 0.1M citrate phosphate buffer (pH 5.0). The homogenate was filtered using the glass fibre filter paper (whatman GF/A) and clarified by centrifugation at 10,000g for 1hr at 4°C. Cellulase activity was determined as described by the method of Miller (1959). The effects of the different concentrations (0, 2, 4, 6 and 8Mm) of each of ethylenediamineacetic acid, mercuric chloride and 2,4 dinitrophenol prepared in 0.6% carboxymethyl cellulose in citrate phosphate buffer (pH 5.0) and the effects of Ca^{++}, K^{+}, Mg^{++} and Na^{+} at concentrations (0mM, 10mM, 20mM, 30mM and 40mM) in 0.6% carboxymethyl cellulose in citrate phosphate buffer (pH 5.0) were examined. The reaction mixture consisted of 1ml of the substrate plus 0.5ml enzyme incubated at 35°C for 1hr and analyzed for cellulase activity as stated above.

Results

During the deterioration of tomato fruits by *A. flavus*, proteins which exhibited cellulase activity were produced. However, similar extracts from the uninfected tomato fruits lacked cellulase activity. The various

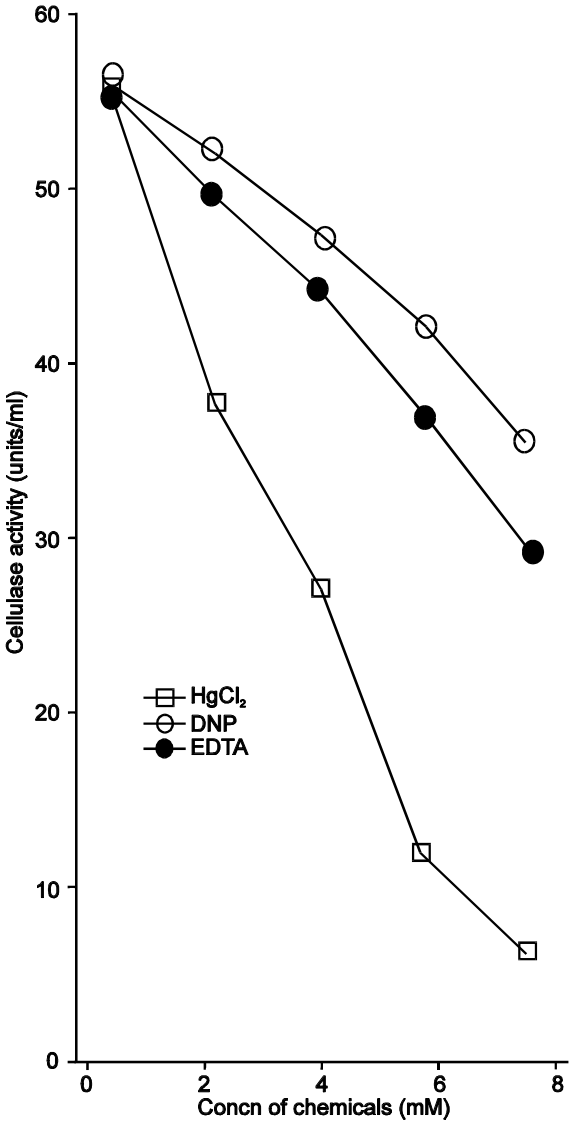

Fig. 1: Effect of chemicals on the activity of partially purified cellulase obtained from tomato fruits deteriorated by *Aspergillus flavus*

concentrations (0mM, 2mM, 4mM, 6mM and 8mM) of ethylenediamine tetraacetic acid (EDTA) 2,4-dinitrophenol (DNP) and mercuric chloride ($HgCl_2$) inhibited the activity of cellulase. The inhibition was higher with increase in the concentration of the chemicals in each case (Fig. 1). Inhibition of approximately 38%, 50% and 90% occurred with 8mM DNP, EDTA and $HgCl_2$ respectively showing that an increase in the concentration of each chemical resulted in a gradual decrease in the cellulase activity of *A. flavus*. The cations employed in this investigation stimulated the activity of the cellulase synthesized by *A. flavus* (Fig. 2). There was a gradual increase in the activity of the enzyme with increase in the concentration of the cations.

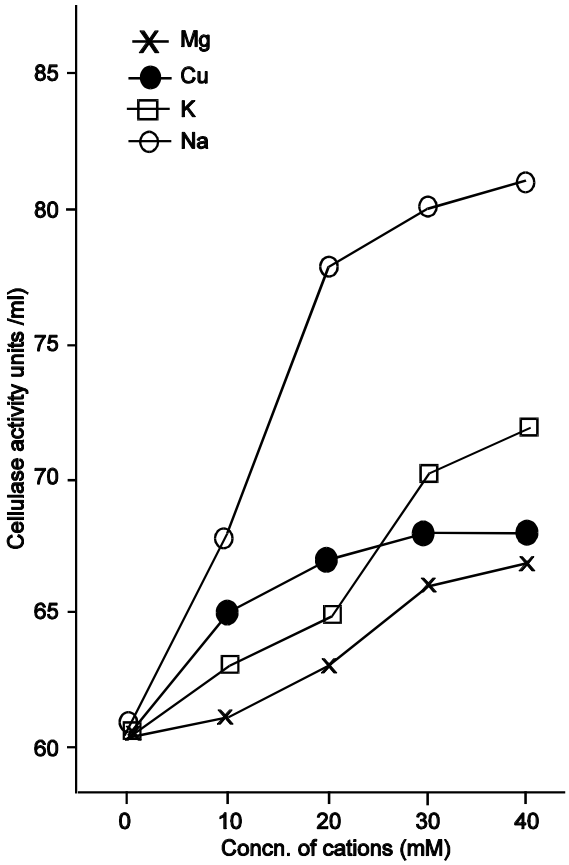

Fig. 2: Effect of cations on the activity of partially purified cellulase obtained from tomato fruits deteriorated by *Aspergillus flavus*

The highest activity was recorded at 40mM cation followed by 30mM cation and the highest stimulation of activity occurred with Na^+ while the lowest stimulation occurred with Mg^{++}.

Discussion

Aspergillus flavus caused extensive degradation of the freshly ripe tomato fruits within ten days of inoculation. Similar results were obtained and reported for the soft rot of tomato caused by *Salmonella Montevideo* (Zhuang and Beuchat, 1996). Extracts from tomato fruits infected by *Aspergillus flavus* exhibited cellulolytic activity. Prabhu and Maheshwari (1999) reported the production of cellulase by *Melanocarpus albomyces*, a thermophilic fungus isolated from compost.

Cellulases from other plant tissues infected by phytopathogens have been repoted (Codner, 1971, Lisker *et al*., 1975 and Ojumu *et al*., 2003). These results therefore suggest that cellulases play an essential role in pathogenicity. The results of this investigation showed that Mg^{++}, Ca^{++}, Na^+ and k^+ were

stimulatory to the activity of the enzyme while small quantities of $HgCl_2$, EDTA and DNP were inhibitory suggests that the enzyme may be cation-requiring (Olutiola, 1982). Stimulation of cellulases by cations as well as their inhibtion by EDTA, DNP and $HgCl_2$ have been reported (Ajayi *et al*., 2003; Oikawa *et al*., 1994; Akiba *et al*., 1995 and Famurewa *et al*., 1993). The results of this research work showed that the uninfected tomato fruits did not possess detectable cellulase whereas tomato fruits infected with *Aspergillus flavus* have appreciable quantity of cellulase activity suggesting that the cellulase is of fungal origin.

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