GLOBAL JOURNAL OF PURE AND APPLIED SCIENCES VOL. 6 NO. 4, SEPTEMBER 2000:619-622
COPYRIGHT (C) BACHUDO SCIENCE CO. LTD. PRINTED IN NIGERIA ISSN 1118-0579

DIGESTION OF CARBON MATERIALS BY PECTOLYTIC AND CELLULOYTIC ENZYMES OF FUSARIUM MONILIFORME SHELD

A. E. ARINZE and A. G. YUBEDEE

(Received 7 September 1999; Revision accepted 4 February, 2000)

ABSTRACT

Apple pectin and sodium polypectate supported the production of polygalacturonases by *Fusarium moniliforme* Sheld while filter paper and carboxymethyl cellulose supported the production of cellulases. Glucose and starch did not support the production of either polygalacturonase or cellulases Reducing sugars were released from sodium polypectate in higher quantity than from apple pectin while more sugars were released from filter paper than from carboxymethyl cellulose by cellulases. Extracellular enzymes from an isolate of *F moniliforme*, Fm-1 released more reducing sugars than another isolate designated Fm-2.

KEY WORDS:- Digestion, polygalacturonase, cellulases, reducing sugars.

INTRODUCTION

The first step in pathogenesis for certain host/pathogen interactions in plants is the extracellular production of pectolytic and cellulolytic enzymes which degrade the complex polysaccharides of the plant cell wall (Bateman and Millar 1966; Wood 1967 and 1973). The production of enzymes is usually inductive and cell wall constituents may serve as inducers of cell wall degrading enzymes. Pectic enzymes play the crucial role of attacking the middle lamella causing a loss of coherence of the host tissues and consequent separation of the cells. This may lead to the release of reducing sugars (Stephens & Wood, 1975).

Some micro-organisms are capable of utilizing native cellulose as carbon source while others use soluble substituted forms of cellulose and produce reducing sugars. This work is aimed at determining the activities of pectolytic and cellulolytic enzymes of *F. moniliforme* in media of different carbon sources and demonstrating the release of reducing sugars from certain synthetic pectic and cellulosic substances.

MATERIALS AND METHODS

Two isolates of *Fusarium moniliforme* designated Fm-1 and Fm-2 were obtained from rotted maize grains and maintained on Potato Dextrose Agar (PDA). Both isolates had white cottony appearance on PDA. Both also had two types of conidia the micro and macro conidia. The micro conidia of isolate Fm 1 were 2 - 3 celled, oval in shape, arranged in chains and measured 8 - 12 x 2.5 - 3 µ. The macro-conidia of Fm 1 were boat shaped, 5 -septate and measured 25 - 60 x 2.5 - µ. on the other hand the micro conidia of Fm2 were also oval in shape but non-septate, borne singly and measured 5 - 12 x 1.5 - 2.5 µ. Also the macro conidia of Fm 2 were sickle shaped but 3-septate measuring 32 - 52 x 3 - 4.5 µ.

CARBON SOURCES: The carbon sources used were apple pectin, cellulose, filter paper, sodium polypectate (Napp), carboxymethyl cellulose (CMC), and starch.

PRODUCTION AND ASSAY OF POLYGALACTURONASE AND CELLULASE IN MEDIA OF DIFFERENT CARBON SOURCES:

The two isolates of *F. moniliforme* were grown in Reese and Levinson's medium (1952) with Napp, apple pectin, CMC, filter paper, glucose and viscometrically using Ostwald-Fenske viscometers, size 300 for polygalacturonase and cellulase activities (Arinze 1985a).

RELEASE OF REDUCING SUGARS: For the release of reducing sugars from pectic substances (Napp and apple pectin) the method of Nelson (1944) as modified by Somogyi (1952) was used while the method of Reese and Mandels (1963) was used to demonstrate the release of sugars from carboxymethyl cellulose and filter paper by cellulases.

RESULTS

Table 1 shows that maximum PG and cellulase activities were obtained for Napp and carboxymethyl cellulose respectively. PG

A. E. ARINZE, Department of Plant Science & Biotechnology, University of Port Harcourt, Port Harcourt - Nigeria
A. G. YUBEDEE, Department of Plant Science & Biotechnology, University of Port Harcourt, Port Harcourt - Nigeria

Table 1. Production of pectolytic and cellulolytic enzymes in media of different carbon sources.

(a) Fm-1

Carbon Source	Polygalacturonase Activity (RVU)	Cellulolytic activity (RVU)
Pectin	121[a]	0
Glucose	0	0
Filter paper	0	156[a]
Sodium polypectate	136[b]	0
Carboxymethyl cellulose	0	160[a]
Starch	0	9

Values are means of triplicate determinations; means with different letters within the same column are significantly different ($P < 0.05$)

(b) Fm-2

Pectin	100[a]	0
Glucose	0	0
Filter paper	0	141[a]
Sodium polypectate	104[a]	0+
Carboxymethyl cellulose	0	134[a]
Starch	0	9

Values are means of triplicate determinations; means with different letters within the same column are significantly different ($P < 0.05$)

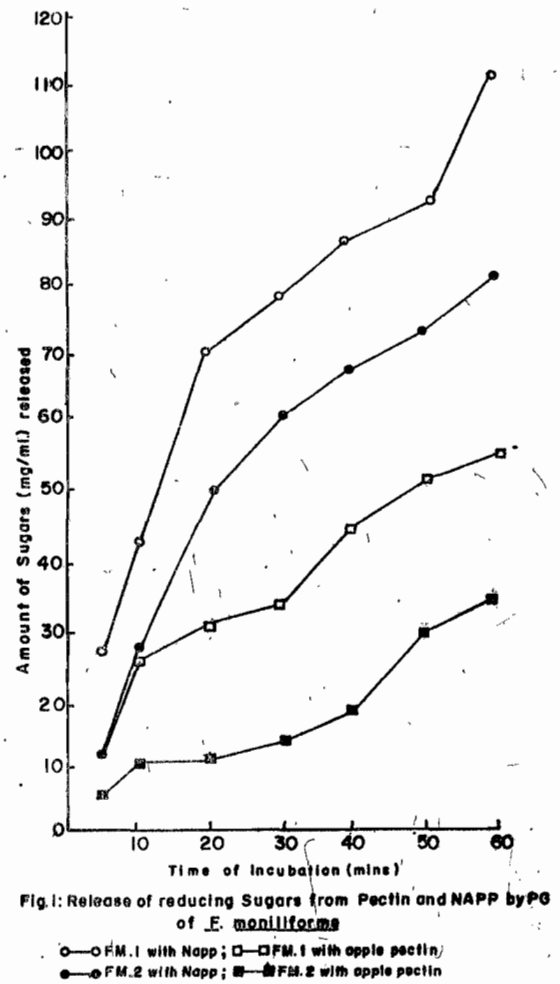

Fig. 1: Release of reducing Sugars from Pectin and NAPP by PG of F. moniliforme

o—o FM.1 with Napp; □—□ FM.1 with apple pectin
●—● FM.2 with Napp; ■—■ FM.2 with apple pectin

activities with Napp as carbon source were 136 and 104 relative viscometric units (RVU) for Fm-1 and Fm-2 repectively. Glucose, filter paper starch and carboxymethyl cellulose did not favour the production of PG enzymes.

Cellulose activity was highest with carboxymethyl cellulose and filter paper. Sodium polypectate, apple pectin, glucose, and starch did not favour the production of cellulolytic enzymes.

Fig. 1 shows that the isolate Fm-1 released more reducing sugars from the carbon materials than isolate Fm-2. Polygalacturonase from Fm-1 which was designated PG1 released 112 and 56ug/ml from Napp and apple pectin respectively. PG2 obtained from the second isolate released 80 and 33μg/ml from the same sources above after 1 hr of incubation.

Fig. 2 shows that cellulose enzymes broke down filter paper and carboxymethyl cellulose to various degrees. After a treatment time of 5 hrs., 120 and 102 ug/ml of reducing sugar were released by cellulase from isolate Fm-1 from filter paper and carboxymethyl cellulose respectively. Also the cellulase from isolate Fm-2 released 101 and 74 ug/ml from filter paper and carboxymethyl cellulose, respectively.

DISCUSSION

A number of carbon sources were tested for their ability to induce the production of pectolytic and cellulolytic enzymes. The suitablility of certain natural products for enzyme production depends on the availability in media of substrates upon which enzymes act. Singh and Wood (1956) reported that *F. moniliforme* produced pectic enzymes in media containing pectic substances. Later, Fanelli and Cervone (1977) reported the production of pectolytic and cellulolytic enzymes in media containing pectin

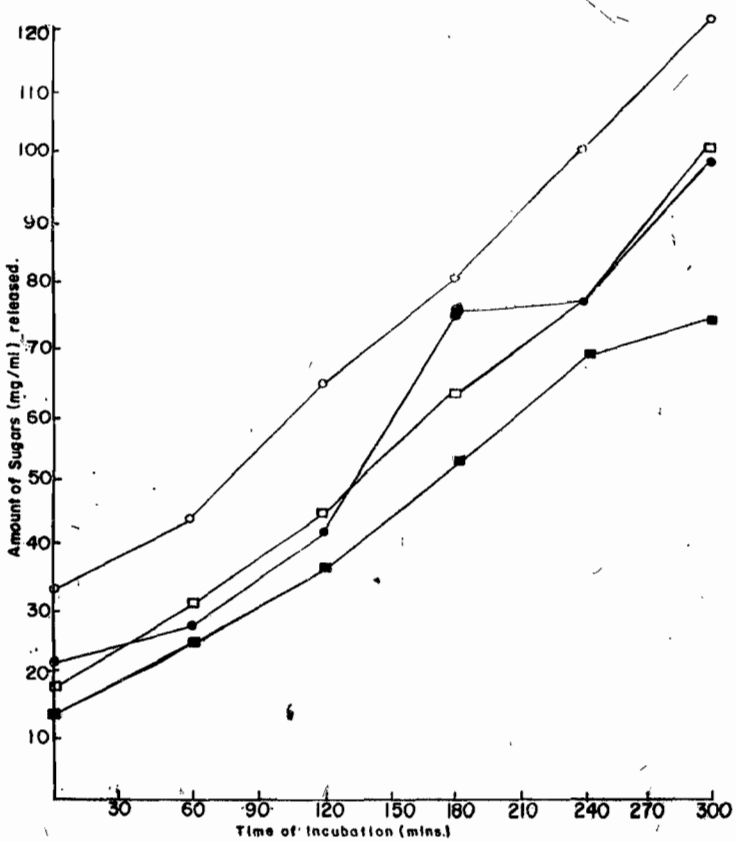

Fig.2: Release of reducing Sugars from filter paper and Carboxymethyl cellulose by cellulase Enzymes of F. moniliforme.
o—o FM. 1 with filter paper; ●—● FM. 2 with filter paper
□—□ FM. 1 with carboxymethyl cellulose; ■—■ FM.2 with carboxymethyl cellulose.

and carboxymethyl cellulose, respectively. They did not report the production of PGs and cellulases in media containing glucose as carbon source. This work showed that only low cellulolytic activity was recorded with starch while PG activity was absent.

Kenkamp *et al* (1952) showed that when pectin was used as carbon source, *Rhizoctonia solani* produced more pectic enzymes than when simple sugars were used. However, Husain and Dimond (1960) reported low cellulase activity in media containing glucose. Bateman (1963) reported low production of cellulases in pectic media and low PG activity in cellulolytic media.

The results, reported here show that with *Fusarium moniliforme* isolate Fm-1, Napp induced the production of PG more than apple pectin and that more reducing sugars were released from Napp than from pectin. However, with isolate Fm-2, enzyme activity was virtually the same with the two carbon sources.

Also cellulases released more reducing sugars from filter paper than from carboxymethyl cellulose. This confirms the report of Wasini (1986) who found that cellulase of *Botryodiplodia theobromae* isolated from Albergine fruits released more reducing sugars from filter paper than from carboxymethyl cellulose. Husain and Dimond (1960) further demonstrated the release of reducing sugars from filter paper, wood cellulose and carboxymethyl cellulose by cellulases of *F. oxysporium* f. sp. *lycopersici*. Oso (1978) showed that *Talaromyces emersoni*, failed to degrade filter paper but its filtrate hydrolysed carboxymethyl cellulose.

The secretion of pectolytic and cellulolytic enzymes in synthetic media by isolates of *F. moniliforme* was adaptive, in that production of a particular type of enzyme occurred only when the media contained favourable carbon substrates. The results also reveal preference for substrates by pectolytic and cellulolytic enzymes. These findings are of particular significance in our further characterization and understanding of the possible roles of these isolates Fm 1 and Fm 2 in the spoilage of maize grains. We now know that polygacturonase and cellulase enzymes produced by FM 1 released more reducing sugars from carbon sources than Fm 2. It may be further deduced that the isolate Fm 1 is more potent as a pathogen than Fm 2. We can also infer that although the two isolates may have a similar pattern of degrading the pectic and cellulolytic components of the tissues, they degrade these substances at different rates. Further purification of the enzymes by isoelectric focusing is being undertaken in our laboratory to further characterise the two isolates. This will provide further information in our understanding of their roles in the pathogenesis of the maize grains.

REFERENCES

Arinze A.E. 1985. Absorption of polygalacturonase isoenzymes of *Botryodiplodia theobromae* on plant tissues and the implication on the pathogenicity of the fungus. Phytopath Zeit 114: 13-20

Bateman, D. F. 1963. Pectolytic activities of culture filtrates of *Rhizoctonia solani* and extracts of Rhizoctonia infected tissues of bean. Phytopathology 53: 197 - 204.

Bateman, D. F. and Millar, R. L. 1966. Pectic enzymes in tissue degradation. Ann. Rev. Phytopathol. 4: 119 - 146.

Fanelli, C. And Cervone F. 1977. Polygalacturonase and cellulase production by *Trichoderma koningii* and *Trichoderma pseudokoningii*. Trans. Br. Mycol. Soc. 68: 291 - 294.

Husain, A. and Dimond, A. E. 1960. Role of cellulolytic enzymes in pathogenesis by *Fusarium oxysporium* f. *lycopersisci*. Phytopathology 50: 329 - 331.

Kernkamp, M. F., Zeeuw, D. J., Chen, S. M. Ortega, B. C., Tsiang, C. T. and Khan, A. M. 1952. Investigations on physiologic specialization and parasitism by *Rhizoctonia solani*. Minn. Agric. Expt. Sta. Tech. Bull. 200, 36p.

Nelson, N. 1944. A photometric adaptation of the Somogyi method for the determination of glucose. Journal of Biochemical Chemistry 153: 375 - 380.

Oso, B. A. 1978. The production of cellulase by *Talaromyces emersoni* Mycologia 70: 577 - 585.

Reese, E. T. and Mandels, M. 1963. Enzymatic hydrolysis of cellulose and its derivatives pp. 139-143. In Methods in Carbohydrates Chemistry vol. III. Cellulose Ed. R. L. Whistler. Academic Press New York.

Singh, R. K. and Wood, R. K. S. 1956. Studies in physiology of Parasitism XXI, The production and properties of pectic enzymes produced by *Fusarium moniliforme* Ann. Bot. N. S. 20: 89 - 103.

Somogyi, M. 1952. Notes on Sugar Determination. J. Biol. Chemistry 195: 19 - 23.

Stephens, G. J. and Wood R. K. S. 1975. Killing of protoplasts by soft not bacteria. Physiological Plant Pathology 5: 165 - 181.

Wasini, A. A. 1986. Survivability and Pathogenicity of *Botryodiplodia theobromae* causing rot of Albergines fruit. Ph.D. thesis University of Port Harcourt.

Wood, R. K. S. 1967. Physiological Plant Pathology. Oxford. Blackwell Scientific Public. Ltd.

Wood, R. K. S. 1973. Specificity in Plant Diseases. In: Fungal Pathogenicity and the Plant Responses. Pp. 1 - 16. Byrde R. J. W. and C. H. Cutting's (eds.) London. Academic Press.