In Vitro And *In Vivo* Production Of Pectic Enzyme, Polygalacturonase, By Seed-Borne Pathogen, *Fusarium moniliforme* Sheldon From Seeds Of Rice (*Oryzae sativa* L) And Its Role In The Diseases Of Rice

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Abstract

Possible *in vitro* and *in vivo* production of pectic enzyme polygalacturonase (PG) by the seed-borne fungal pathogen *F moniliforme*, and the effect of temperature and pH on the activity of the enzyme were investigated .The result of the assay for the production of polygalacturonase (PG) by the pathogen showed that the activity was 142.9 RVU *in vitro* and 166.7 RVU, *in vivo* at temperature 25 °C .The optimum activity of the enzyme *in vitro* was obtained at 25 °C and 30 °C, with the value of 142.9 RVU, and the least was at 20°C, 45 °C and 50 °C, with a value of 111.1 RVU. *In vivo*, the optimum activity was at 25 °C, with a value of 166.7 RVU, while the least activity was 111.1RVU at 50 °C. The activity of PG in vitro was optimum at pH 5 and 6, with a value of 142.9 RVU each, while the least activity was obtained at pH 2, 9 and 10, with a value of 111.1 RVU. During the *In vivo*, the activity of PG was optimum at pH 6 with a value of 166.7 RVU, and least at pH 2, 9, and 10, with a value of 111.1 R.VU.

Keywords: Enzyme activity, Polygalacturonase, Optimum Temperature, *Fusarium moniliforme, Oryzae sativa*

Introduction

Polygalacturonase (PG) E.C 3.2.1.15 is a hydrolytic enzyme which breaks down pectic substances of the middle lamella. The polygalacturonase breaks down the polygalacturonide chains of the α-1, 4 - glycosidic linkage to produce shorter chains and reducing groups. Several investigators have demonstrated that this pectic enzyme is produced *in vitro* and *in vivo*, though, according to (Akanu-Ibiam and Arinze, 1999), it is produced inductively rather than constitutively, in most cases. Fergus and Wharton (1975) reported that pectic substances are the main inducers of pectic enzymes in culture media. Arinze and Smith, 1979) and (Arinze, 1985a) reported that polygalacturonase was produced *in vitro* and *in vivo* by *Botryodiplodia theobromae* in potato tissue. Akanu-Ibiam and Arinze, (1999) also reported that it was produced by *Fusarium moniliforme* isolated from carrot tissues, *in vitro*. Barker and Walker, (1962) reported that the optimum temperature for the activity of PG from *Pellicularia filamentosa* (Pat) was in the range 24-28 °C, while Akanu-Ibiam and Arinze, (19991), reported that it was 25 °C for that secreted by *Fusarium moniliforme in vitro* .Singh and Wood (1956), reported that the optimum pH for the activity of PG was pH 8 – 9. This was in contrast to the report of (Bateman and Millar, 1966), who reported that its pH was near pH 6 and by (Barmore and Brown, 1981; Akanu-Ibiam and Arinze, 1999; Gao and Shain, 1995) who reported that the optimum pH for PG activity was pH 5.

The major aim of this research is to investigate the production of this pectic enzyme by the seed-borne pathogen of rice, *Fusarium moniliforme* Sheldon, and study the effects of temperature, and pH on the activity of these enzymes, and their possible involvement in seed-borne diseases of rice.

Materials and Methods

For the study of polygalacturonase *in vitro*, the method of Reeze and Levinson (1952) was used. 25 ml of medium containing 10 g of pectin, 4.6 g. $NaNO_3$, 1.0 g KH_2PO_4, 0.5 g $MgSO_4$ $7H_2O$ and 0.1 g yeast extract per litre of distilled water was introduced into 250 ml Erlenmeyer conical flasks.5mm disc of five-day old culture of *Fusarium moniliforme*, was

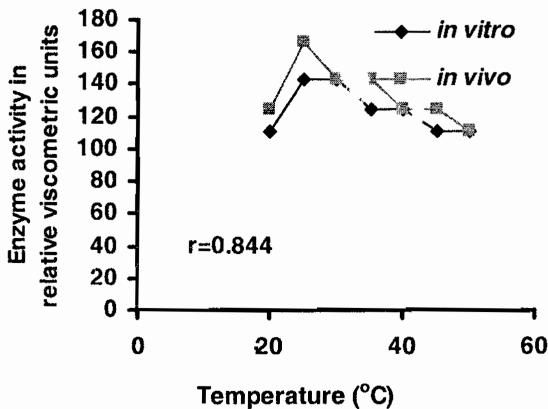

Fig. 1. Effect of temperature on the activity of polygalacturonase secreted by *Fusarium moniliforme in vitro* and *in vivo*

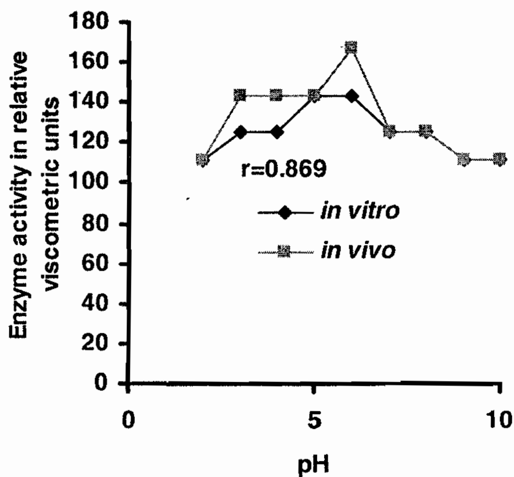

Fig.2. Effect of pH on the activity of polygalacturonase secreted by *Fusarium moniliforme in vitro* and *in vivo*

introduced into each flask. These were incubated for four days at 25 °C. Three replicates were made. After four days, the enzyme filtrate of the pathogen was obtained by removing the mycelia and filtered with two layers of sterile muslin cloth.

For the *in vivo* studies, homogenates were obtained by removing the tissues rotted by the test fungal pathogen, with sterile carpel. Following the method of (Arinze, 1985a), the rotted tissues were mixed with 0.1M phosphate buffer pH 7.0 (1 g tissue/10ml buffer) containing 0.2 M NaCl (to de-absorb proteins from the tissues), and 0.001M ascorbic acid (to prevent oxidation).The extract was prepared by homogenizing the tissues in a sterile Warring blender, and straining the homogenate through sterile muslin cloth.

The method of (Spalding, 1966), was used to purify enzymes from culture filtrates of the test fungal pathogen .The filtrate was centrifuged at 2,500 g for 15 minutes, and the deposited insoluble compounds discarded. Cold acetone was added to the supernatant to precipitate the protein fraction. The precipitate was collected by centrifuging at 2,500 g for 15 minutes, and then dissolved in 0.1M phosphate buffer at pH 6.0.Fresh enzyme preparation was made on the day of each experiment. The precipitate was used for enzyme studies.

Activity of polygalacturonase from the pathogen was assayed by the use of 300 size Oswald Cannon Fenske viscometer. This method is routinely used to determine the chain splitting reaction of culture filtrates and homogenates. The reaction mixture contained 4ml of 1% pectin in 0.1M citrate buffer pH 5.0, 1 ml of water and 2ml of enzyme sample. Enzyme activity was expressed in viscometric units, defined as 1000/t, where t = time in seconds for 50% loss in viscosity of the reaction mixture (Arinze, 1985a and Arinze, 1985b) at 25 °C, in a water bath. Viscometers were calibrated against water. The flow time for water represented 100% loss in viscosity.

The effect of temperature on the activity of PG from each of the fungal pathogens was investigated. Samples of the reaction mixtures, which were the same as described earlier in the viscometric assay was used at 20 °C, 25 °C, 30 °C, 40 °C and 50 °C in a water bath for ten minutes. The activity of the enzyme was determined viscometrically.

The effect of pH on the activity of PG from the four fungal pathogens was tested at nine pH levels 2, 3, 4, 5, 6, 7, 8, 9 and 10 using citrate, potassium chloride and boric acid buffer solutions prepared as described by (Hale, 1958).The reaction mixture was the same as that used to test for viscometric activity of PG. The reaction mixture at each level was incubated at 25 °C for 10 minutes, after which the enzyme activity was determined viscometrically.

Result

The result of the assay for the production of the pectic enzyme polygalacturonase by *Fusarium moniliforme*, showed that the

activity of the enzyme when tested viscometrically, using 10 % pectin was 142.9 RVU (Relative Viscometric Units) *in vitro*, while it was 166.7 RVU *in vivo* at 25 °C.

As shown in Fig.1, the optimum activity of the enzyme secreted by *F moniliforme,* was obtained at 25 °C and 30 °C, with the value of 142.9 RVU, and the least was at 20 °C, 45 °C and 50 °C, with a value of 111.1 RVU. The activity at each of the other temperatures was 125 RVU at 35 °C and 40 °C each, *in vitro. In vivo*, the activity of PG produced was optimum at 25 °C, with a value of 166.7 RVU, while the least activity was 111.1 RVU at 50 °C. The activity of the enzyme at each of the other temperatures was 142.9 RVU at 30 °C and 35 °C, and 125 RVU at 20 °C, 40 °C and 45 °C each.

As shown in Fig.2, the activity of PG secreted by *F moniliforme in vitro* was optimum at pH 5 and 6, with a value of 142.9 RVU each, while least activity was obtained at pH 2, 9 and 10, with a value of 111.1 RVU each. The activity of the enzyme at each of the other pH levels was 125 RVU at pH 3, 4, 7 and 8 *in vitro. In vivo,* the activity of PG secreted was optimum at pH 6 with a value of 166.7 RVU, and least at pH 2, 9, and 10, with a value of 111.1 R.VU. Its activity at each of the other pH levels, pH 3, 4 and 5 was 142.9 RVU each, and 125 RVU at pH 7 and 8.

Discussion

The activity of the enzyme polygalacturonase secreted by *Fusarium moniliforme* in *vitro* and *in vivo* was assayed at 25 °C, and the activity of PG from the pathogen *in vitro* was 142.9 RVU, whereas the activity *in vivo* was 166.7 RVU. This enzyme might have been involved in decay of the seed coat of the seeds of these varieties, making it easy for the penetration of the pathogen, to reduce the nutrients or cause seed rot. When these seeds are planted, it might, perhaps, be involved in the maceration of tissues of the crop both in the nursery and field, pre-disposing them to attack by other agents which could bring about synergism of infection and resultant damage.

According to (Arinze and Smith, 1979), PG was secreted in infected tissues by *Botryodiplodia theobromae* and in seedling cell walls of wheat by *Rhizoctonia cerealis.* It was reported to have been secreted by *Aspergillus niger* by Cervon *et al*, (1978), and by *Penicillium italicum* Wehmer (Hershenhorn *et al*, 1990). Cooper *et al*, (1990), reported that it was secreted by *Fusarium culmorum* and *Pseudocercosporella herpotrichoid.* It was also secreted in infected leaves of rice by *P oryzae* (Prabakar, 1991); in infected tomato plant by *Fusarium oxysporum. fsp .lycopersici* (Anthonio and Rancero, 1996); and in *Vigna ungunculata* Walp, by *F moniliforme* (Capari *et al*, 1990).Akanu-Ibiam and Arinze (1999), reported that PG from *F moniliforme in vitro*, was involved in cutical decay of carrots.

The optimum temperature for the activity of PG from *F moniliforme* was 25 °C and 30 °C *in vitro* and 25 °C *in vivo.* There was significant difference in the activity of the enzyme secreted *in vitro* and *in vivo* $r < 0.01$ (Fig.1). The decreased activity of PG with increase in temperature for both *in vitro* and *in vivo* studies, suggests that the enzymes secreted under the two conditions were different. The temperature optimum for PG *in vitro* was 25 °C (Akanu-Ibiam and Arinze (1999), and in the range 24-28 °C for those from *Pellicularia filamentosa* (Pat) (Barker and Walker, 1962).The decrease in the activity of these enzymes as a result of increase in temperature, indicated that the enzymes were being denatured, hence, decrease in their activity. Wiseman and Gould (1971) stated that influence of temperature on the activity of enzymes was due to the effect on the stability of the enzyme and the enzyme substrate breakdown velocity.

The optimum pH for the activity of the PG from the pathogen was acid *in vitro* and *in vivo.* The optimum pH for the activity of the enzyme was pH 5 and 6 *in vitro* and pH 6 *in vivo* (Fig.2).There was significant difference in the activity of the enzyme secreted by the pathogen *in vitro* and *in vivo* $r < 0.01$.This suggests, perhaps, that the enzyme secreted *in vitro* and *in vivo* were different. This result is in contrast with the report of (Singh and Wood,1956) that the optimum pH for the activity of PG was pH 8-9, but supports the report of (Bateman and Millar,1966) that its pH is near pH 6, and by (Barmore and Broun,1981, Gao and Shain, 1995, and Akanu-Ibiam and Arinze,1999), that the optimum pH for PG activity was pH 5. However, the results obtained here could indicate, perhaps, that the pH optimum for the activity of polygalacturonase from rice

mold is acid, pH 5 and 6 and that below or above this pH, their activity would not be very effective or could lead to de-naturation. The effect of pH on enzyme activity could be explained in terms of the relative molecular stability of the enzymes (Lehninger,1973), and partly on the state of ionization of the substrate, enzymes, or enzyme-substrate complex as the pH changes (Zefere and Hall, 1973).The ability of the pathogen to secrete this enzyme *in vivo* has proved that it could aid in seed damage, and attack of the crop and cause disease in the field. This is because pectic enzymes though secreted *in vitro* cannot be claimed to be involved in the damage of the seeds of the crop or cause disease in the field until it is isolated *in vivo,* as reported by (Bateman, 1963, Hancock, 1965, and Bateman and Beer, 1965).

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