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SHORT COMMUNICATION

PECTOLYTIC ENZYMES IN THE DEVELOPMENT OF *COLLETOTRICHUM* LEAF DISEASE IN RUBBER *(HEVEA BRASILIENSIS)*

LILANI K. SENARATNA*, R.L.C. WIJESUNDERA
Department of Botany, University of Colombo, Colombo 3.

AND

A. DE S. LIYANAGE
Rubber Research Institute, Dartonfield, Agalawatte.

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Abstract: A pectin lyase was detected in **Hevea brasiliensis** leaves infected with **Colletotrichum gloeosporioides.** Pectin lyase activity was first detected on the third day after inoculation and the activity increased rapidly thereafter. This increase was associated with the development of lesions. Polygalacturonase (PG) activity was not detected at any stage of the infection. Inhibition of PG by leaf tissue or binding of PG to host tissue was also not detected.

Key words: Colletotrichum gloeosporioides, Hevea brasiliensis, pectin lyase, rubber.

INTRODUCTION

Colletotrichum leaf disease of rubber [*Hevea brasiliensis* (Willd. ex Juss.) Muell. Arg.] is caused by the fungus *Colletotrichum gloeosporioides* Penz.. The disease is common in Sri Lanka and the symptoms include lesions on leaves, distortion of leaves and young shoots, and sometimes premature defoliation.[1] Isolates of the fungus *C. gloeosporioides* are known to secrete the pectolytic enzymes, polygalacturonase (PG ; E.C.3.2.1.15) and pectin lyase (PL ; E.C.4.2.2.10) and the cellulolytic enzymes β-1,4 glucanase and β-glucosidase when grown in liquid media.[2] Studies on pectolytic enzymes from several other pathogens have shown evidence to support the involvement of these enzymes in pathogenesis.[3] We report here on the role played by pectolytic enzymes secreted by the fungus in the development of disease in leaves of *H.brasiliensis.*

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* Present Address: Botany Division, Open University, Nawala, Nugegoda.

METHODS AND MATERIALS

Host Plant: *Hevea brasiliensis* clone 121 from the budwood nursery of the Rubber Research Institute provided leaves for all experiments.

Organism: Isolate 5 of *Colletotrichum gloeosporioides* was obtained from the Plant Pathology Department of the Rubber Research Institute. Stock cultures were maintained at 4°C on potato dextrose agar (PDA).

Preparation of Inoculum: Fifteen ml of PDA medium was dispensed into 150 ml Erlenmeyer flasks and the medium inoculated with *C. gloeosporioides,* and incubated as described by Senaratna et al[2]. When the cultures were 7d old, 10 ml of sterile distilled water was added to each flask and shaken for 5-10 minutes. The spore concentration of the resultant suspension was adjusted to $2x10^5$ spores ml^{-1} using a haemocytometer and the suspensions were then filtered through four layers of muslin. The resulting filtrate was used to inoculate *H. brasiliensis* leaves.

Inoculation: Young *H. brasiliensis* leaves at the copper brown stage of development were inoculated by placing the fungal suspension (0.05 ml) on the abaxial surface. The cut ends of the petioles were then sealed with molten wax, and the leaves incubated at 30 ± 1°C in moist, large petri dishes (15 cm in diameter). As controls, leaves were inoculated with sterile distilled water (0.05 ml).

Secretion of Polygalacturonase (PG): To obtain PG, the fungus was grown in the ammonium tartrate liquid medium described by Byrde and Fielding[4] with citrus pectin as the main source of carbon, and inoculation, incubation and harvesting were carried out as described by Senaratna *et al*..[2]

Tissue Extraction: The inoculated leaves were harvested at 24 h intervals for 6 days, and were stored at -4°C until used. Initially acetone treated leaf powder was prepared from leaf tissue according to the procedure of Wong *et al.*[5] Thereafter the enzymes were extracted from the acetone powder with 0.01 M Tris-HCl buffer (pH 7.6) as described by Batra and Kuhn.[6] The extract was stored at -4°C and used to detect the presence of cell wall degrading enzymes. For some experiments the extracts were treated with 5% solution of NaCl in 0.01 M Tris-HCl (pH 7.6) to desorb enzymes bound to cell walls.[4,7]

Determination of Cell Wall Degrading Enzyme Activity: The agar plate method[8] was used to determine PG activity. Activities were expressed relative to an aqueous solution of Pectinol 10 M (1.0 mg ml^{-1}) which was defined as having 100 units of PG activity ml^{-1}.[4]

PL was assayed using the thiobarbituric acid (TBA) method.[9] One unit of enzyme activity is defined as that amount which produces 1 μmol of unsaturated uronide in 1 min based on the molar extinction coefficient for the product of 4600.[10]

Determination of Molecular Weight: The approximate molecular weight of the pectolytic enzyme was determined by gel filtration,[11] using a column of Sephadex G-100 eluted with 0.05 M Tris-HCl buffer having 0.1 M potassium chloride and 0.2 N sodium azide. Bovine serum albumin, ovalbumin, haemoglobin (monomer), lysozyme and cytochrome c (Sigma, St. Louis, U.S.A.) were used as marker proteins.

Effect of Host Tissue on Polygalacturonase: The ability of both healthy and infected leaf extracts to inhibit the polygalacturonase produced by *C. gloeosporioides* was examined as described by Fielding[12]. The enzyme used for the assay and the leaf extracts were prepared as described earlier. A mixture of enzyme and the potential inhibitor in the proportions 1:9; 3:7; 1:1; 7:3; 9:1 (v/v) were added to wells in agar plates followed by incubation and development. In control experiments sterile distilled water was used instead of leaf extract.

RESULTS

Pectolytic Enzymes in Leaf Tissue: PL was the only pectolytic enzyme detected in extracts of *Hevea* leaves infected with the fungus. The PL activity was first detected on the third day and the activity reached a maximum on the fifth day after inoculation (Figure 1). PG was not detected at any stage of the infection. No pectolytic enzyme activity was detected in leaf extracts from healthy leaf tissue at any stage. Leaf extracts treated with 5% NaCl also did not possess any PG activity.

Molecular Weight: PL detected in infected leaf tissue was eluted after cyclochrome C on G-100 gel filtration suggesting a mol.wt. < 12,000 (data not shown).

Effect of Leaf Tissue on PG Activity: Extracts of both healthy and infected leaf tissue did not have any significant influence on the activity of PG produced by *C. gloeosporioides* (Table 1).

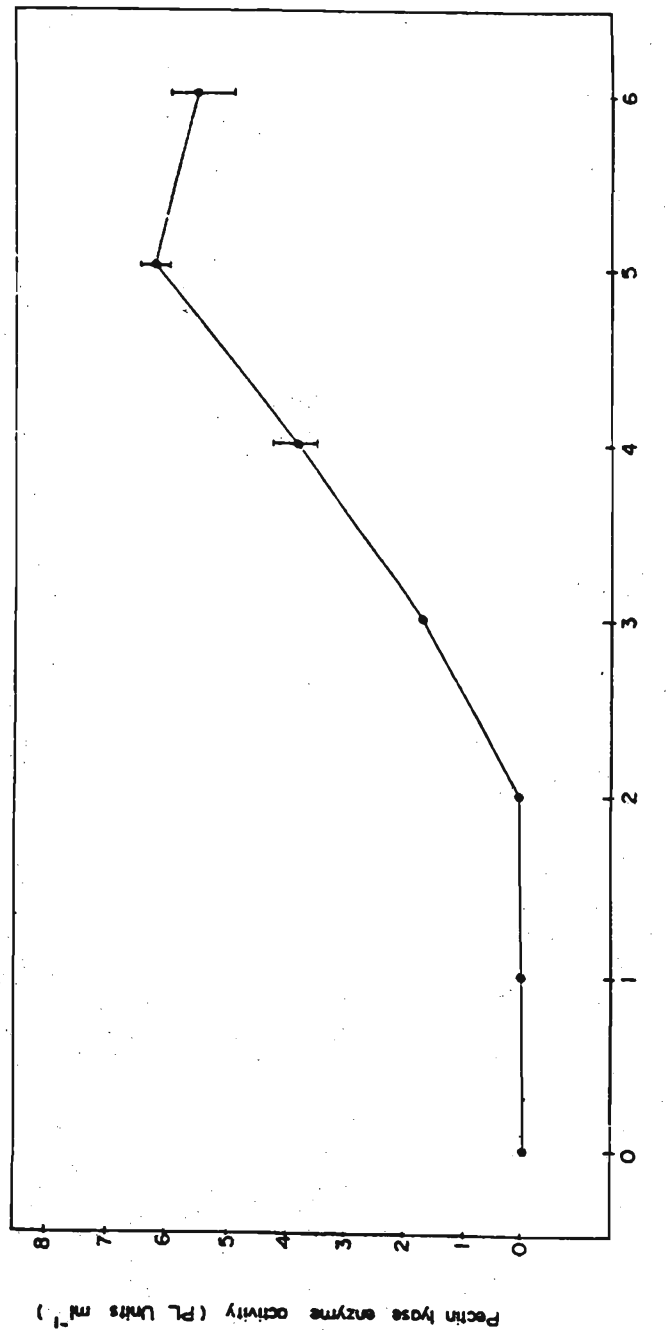

Figure 1: Pectin lyase enzyme activity in Colletotrichum gloeosporioides infected Hevea leaf extract. (S.E. is indicated by vertical bars)

Table 1: Effect of *Hevea* leaf extracts on Polygalacturonase (PG) activity of *C. gloeosporioides*.

	Diluent		
Dilution of Enzyme diluent:enzyme (v:v)	Healthy leaf extract	Infected leaf extract PG activity (PG Units)	Sterile distilled water
9 : 1	253 ± 3	255 ± 2	255 ± 2
7 : 3	635 ± 7	640 ± 5	641 ± 4
1 : 1	1596 ± 12	1608 ± 10	1608 ± 10
3 : 7	2530 ± 30	2535 ± 25	2538 ± 26
0 : 10	3981 ± 0	3981 ± 0	3981 ± 0

DISCUSSION

Liyanage and de Alwis[13] showed that the entry of *C. gloeosporioides* into leaf tissue occurs about 9 h after inoculation and colonization takes place 48 h after inoculation. Lesions are produced thereafter. The increase in PL activity in host tissue therefore coincides with fungal colonization and lesion formation. PL detected in infected tissue had a mol.wt. < 12,000 which is compatible with that of PL secreted by *C. gloeosporioides* in culture[2]. Since PL activity was not detected in healthy leaves the evidence strongly suggests that the PL detected in infected leaf tissue is of fungal origin.

Many plant pathogens secrete PG very early in infection.[7] *C. gloeosporioides* secreted PG in culture [2] but PG activity was not detected at any stage of the infection. A similar phenomenon occurs with the pathogen *Colletotrichum lindemuthianum*[14] and *Cladosporium cucumerinum*.[15] Both *C. lindemuthianum* and *C. cucumerinum* produce PG in culture, but not in infected tissue. In both, binding of the enzyme to cell walls and the presence of a PG inhibitor in host tissue has been cited as the likely causes for the failure to detect PG activity.[14] In the present investigation PG activity was not detected even after host tissue was treated with 5% NaCl and both infected and healthy host leaf tissue had no effect on the PG secreted by *C. gloeosporioides* in culture. Thus, binding of PG to host cell walls or the presence of a PG inhibitor in leaf tissue appears unlikely.

The results are consistent with the suggestion that the PL produced by *C. gloeosporioides* plays a major role in the development of the *Colletotrichum* leaf disease of *H. brasiliensis*.

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