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DIFFERENCES BETWEEN THREE *PORIA HYPOLATERITIA* ISOLATES IN SRI LANKA.

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Abstract: Three isolates of *Poria hypolateritia*, the fungus causing the red root disease in the tea plant, were obtained from three climatically different areas of Sri Lanka and examined for differences in growth and secretion of enzymes involved in pathogenesis. Significant differences between the growth rates of the isolates were seen in many growth media. All isolates showed optimum growth at 25° C, but the optimum pH for growth differed among the isolates. All three isolates secreted a single form of polygalacturonase. Two of the isolates secreted three forms of ß-glucosidase that differed in molecular weights while one isolate secreted only two forms. The results show that significant differences exist among the three isolates of the fungus.

INTRODUCTION

Poria hypolateritia causes the red root disease of tea. Red root is a serious root disease of tea in Sri Lanka and the disease occurs at elevations between about 750 m and 2,000 m.[1] The elevations between 750 m and 2,000 m include regions with different climatic conditions.

Differences among isolates of fungal pathogens of rubber obtained from different regions of Sri Lanka have been reported.[2,3] A knowledge of the variation in pathogenic fungi is useful for disease control, development of new clones or cultivars and in cultivation programmes. There is no information on variation in *P. hypolateritia* in Sri Lanka. This investigation was initiated to determine whether significant differences exist among isolates of *P. hypolateritia* originating from regions with different climatic conditions. Isolates of *P. hypolateritia* were obtained from three such regions and the growth of the isolates and secretion of two enzymes *viz.* polygalacturonase and β - glucosidase, that have a role in pathogenecity,[4] examined.

METHODS AND MATERIALS

Fungal Isolates - Three isolates of *P. hypolateritia* obtained from the Tea Research Institute, Talawakalle, were used in this investigation. The isolates were T 1 (from

Hettikanda, Ratnapura; altitude 1,066 m; soil pH 4.3), T 2 (from Densford, Nanu-Oya; altitude 1,505 m; soil pH 4.4) and T 3 (from Madulkelle, Kandy; altitude 1,371 m; soil pH 5.1). The isolates were maintained on potato dextrose agar at 4° C during the period of study (about 3 months).

Media - (a) Solid Media. Potato dextrose agar (PDA), Malt extract agar (MEA), Corn meal agar (CMA), Czapek-Dox agar (CDA) and Root extract agar (REA) were used. REA was made from 35 g of powdered, dried, healthy tea roots and 15g agar in 1 l distilled water. Fifteen ml of each medium was dispensed in 9 cm diameter petri dishes.

(b) Liquid Media. The buffered Czapek - Dox medium without agar was prepared by adding 100 ml of 0.2M citric acid phosphate buffer to 100 ml double strength Czapek-Dox medium.

For enzyme secretion studies the ammonium tartrate medium[5] was used. Citrus pectin was the main source of carbon for polygalacturonase secretion studies and carboxy methyl cellulose for studies on ß - glucosidase. Twenty five ml of liquid medium was dispensed in 150 ml Erlenmyer flasks.

Inoculation and Incubation - The solid media were inoculated at the centre with plugs of 0.5 cm diameter taken from the periphery of 8-day old cultures of each isolate of the fungus on PDA at 25° C. Unless otherwise stated all inoculated cultures were incubated at 25° C. The liquid media were inoculated with two 0.5 cm diameter plugs taken from cultures as above. The inoculated media were incubated at 25° C without shaking.

Assessment of Growth - Growth on PDA, MEA, CMA, CDA and REA were assessed by measuring the colony radius along two axes at right angles 48, 72, 96,120, 144, 166 and 196 h after inoculation as described by Jamil and Nicholson.[6]

Effect of Temperature on Growth - To examine the temperature effect the isolates were grown on PDA and incubated at 15°, 20°, 25°, 30° and 35° C and growth assessed as above.

Effect of pH on Growth - The isolates were grown on Czapek-Dox liquid medium buffered at pH values 3.0, 3.5, 4.5 , 5.5 and 6.5. To assess growth cultures were harvested at 48 h intervals for 16 d by filtering through Whatman no.1 filter paper and the residual mycelium was dried to a constant weight at 80°C in a hot air oven for 6-8 h and the weight was determined.[2]

Determination of Enzyme Activity - The liquid media were harvested 12 d after inoculation by filtration through Whatman no.1 filter paper. The culture filtrates were dialysed against distilled water at 4° C for 48 h and concentrated ten fold by freeze drying . The dialysed, concentrated culture filtrates were used for enzyme assays.

Polygalacturonase (PG): Polygalacturonase activity was determined using the agar plate method and by the release of reducing sugars from 0.1% (w/v) solutions of polygalacturonic acid in 0.1M sodium acetate buffer (pH 5.0)[2]. In the agar plate method a sodium polypectate agar gel was used and activities were determined relative to an aqueous solution (1 mg/ml) of pectinol 10M (Rhom & Hass, USA). β-Glucosidase: β-Glucosidase activity was measured by the hydrolysis of the chromogenic substance p-nitrophenyl β-D-glucopyranoside.[5] The p-nitrophenol released were estimated by measuring the absorbance at 403 nm.

Determination of Molecular Weights - Molecular weights were determined by gel filtration[7] using a column of Sephadex G-100 (80x2.6 cm^2) eluted with 0.05M Tris-HCl buffer of pH 7.6 having 0.1M potassium chloride and 0.2N sodium azide. Bovine Serum Albumin(66,000), Egg Albumin(45,000), Pepsin(34,700) and Cytochrome - c(12,400) (all from Sigma, St. Louis, USA) were used as marker proteins.

RESULTS

On REA and CMA the growth rate of T 1 was significantly different from the other two isolates and on MEA and CDA the growth rates of all isolates differred significantly from each other (Table 1). There was no significant difference among the growth rates of the three isolates on PDA.

Table 1: The growth rates of isolates of *Poria hypolateritia* on different media.

Isolate	Growth.rate (cm/day)*				
	REA	MEA	CDA	PDA	CMA
T 1	0.35 a	0.59 a	0.45 a	0.74 a	0.58 a
T 2	0.24 b	0.78 b	0.32 b	0.79 a	0.69 b
T 3	0.22 b	0.95 c	0.59 c	0.75 a	0.69 b

* Mean of 5 replicates. Values followed by the same letter in a column are not significantly different at P=0.05 (Duncan's multiple range test). REA- root extract agar, MEA- malt extract agar, CDA- Czapek-Dox agar, PDA- potato dextrose agar, CMA- corn meal agar.

The isolate T 1 had optimum growth at pH 4.0, T 2 at pH 3.5 and T 3 at pH 5.0 when grown in Czapek-dox liquid media (Fig. 1). The growth was optimum at 25° C for all isolates. There was a rapid decrease of growth above 30° C and below 20° C in all three isolates (Fig. 2).

All three isolates secreted polygalacturonase when grown in liquid media with pectin. The molecular weights of the enzymes were: T 1 = 32,000 ± 800 ; T 2 = 33,200 ± 750 ; T 3 = 32,500 ± 500 (The results are the mean of three replicates, ± standard errors).

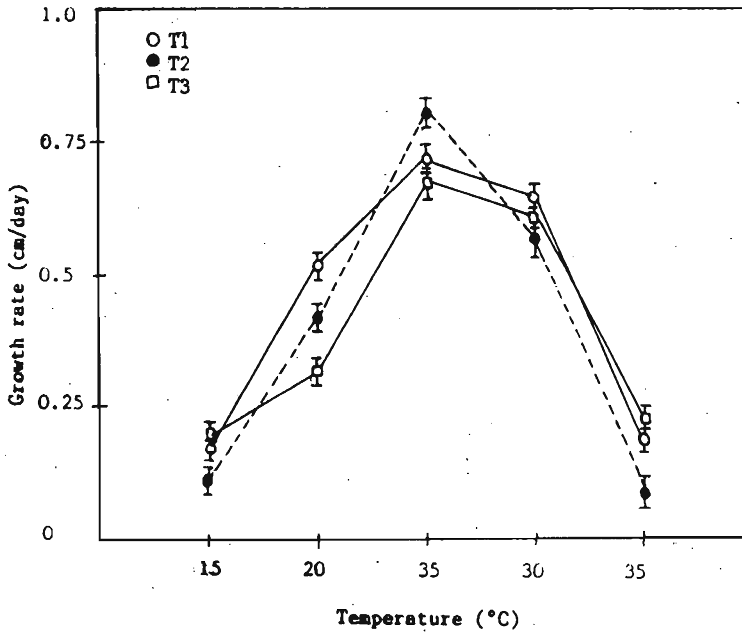

Figure 1: Growth of *Poria hypolateritia* isolates at different temperatures. Values are the mean of three replicates. Vertical bars indicate standard errors of the mean.

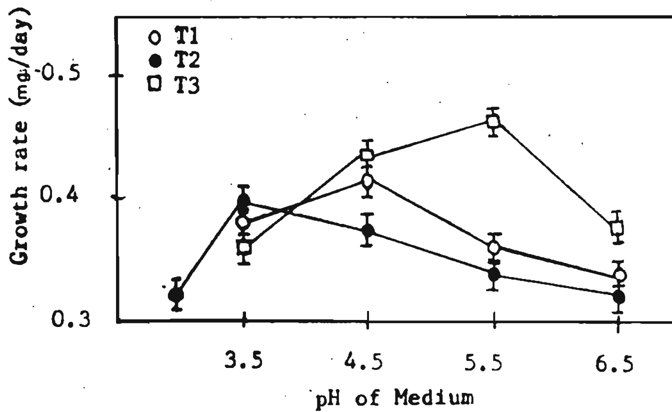

Figure 2: Growth of *Poria hypolateritia* isolates in Czapek-Dox liquid medium buffered at different pH values. All values -mean of three replicates. Vertical bars indicate standard errors of the mean.

Only two forms of ß-glucosidase were detected in T 1 whereas in both T 2 and T 3 three forms were detected. The molecular weights were:

T 1 = 25,000 ± 500 ; 34,500 ± 700.
T 2 = 23,750 ± 750 ; 35,000 ± 300 ; 52,000 ± 700.
T 3 = 24,000 ± 950 ; 36,500 ± 800 ; 53,000 ± 500.
(All values - mean of three replicates, ± standard errors).

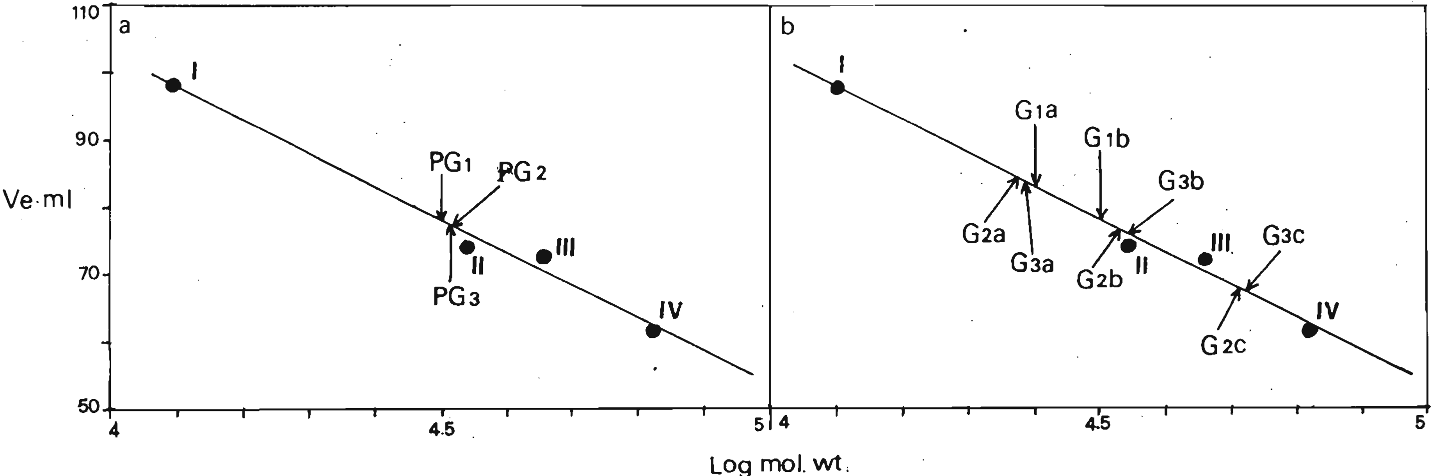

Figure 3: Elution profiles of (a) polygalacturonase, PG and (b) β-glucosidase secreted by the isolates of *P. hypolateritia*.

I Cytochrome-c
II Pepsin
III Egg albumin
IV Bovine serum albumin

PG 1[32,000], PG 2[33,200], PG 3[32,500] are polygalacturonases secreted by isolates T 1, T 2 T 3 respectively.
G1 a[25,000], and G1 b[34,500] are T 1 β-glucosidases.
G2 a[23,750], G2 b[35,000] and G2 c[52,000] are T2 β-glucosidases.
G3 a[24,000], G3 b[36,500] and G3 c[53,000] are T3 β-glucosidases.

The elution profiles of the enzymes are given in Figure 3.

DISCUSSION

The growth rate of T 1 was always significantly different from T 2 and T 3, except when grown on PDA. The cause for the variation in growth rates in different media is likely to be the availability of nutrients. Synthetic media such as PDA are rich in nutrients and the nutrients are easily available to the fungus, hence the higher rates of growth. In REA where the rates of growth were slower the availabilty of nutrients is relatively low.

The isolates had differnt pH optimas for growth. It is of interest to note that in isolates T 1 and T 3, the pH of the soil from which the isolates were obtained and their pH optimas for growth match ; T 1 pH optima 4.5 (soil pH 4.3), T 3 pH optima 5.5 (soil pH 5.1). This could be an adaptation of the isolates to their environments. However, this was not observed in T 2 where the pH optima was 3.5 and soil pH 4.4. The temperature optimum for growth did not differ among the isolates.

The molecular weights of the enzymes suggest that, (a) All isolates secrete a similar form of polygalacturonase. (b) The isolates T 2 and T 3 secrete three similar forms of β-glucosidase whereas T1 secrete only two of these forms.

It can be concluded that significant differences exist among the three isolates studied. The isolate T 1, which was obtained from the lowest elevation, differred most ie. in the rate of growth, pH optima and secretion of β-glucosidase. The isolates T 2 and T 3 differed from each other in the rate of growth on two media and in pH optima.

The detection of these isolates with different characteristics must be taken into consideration in the future in disease control, developing new clones and planting programmes of the tea industry in Sri Lanka.

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