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A STUDY ON TOXIN PRODUCED BY XANTHOMONAS CAMPESTRIS PV. ARECAE INCITANT OF BACTERIAL LEAF STRIPE OF ARECANUT

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ABSTRACT : *Xanthomonas campestris* pv *arecae* causal agent of Bacterial leaf stripe disease of arecanut produced 2 g/l of extracellur toxic polysaccharide, a dilute (0.2 per cent) aqueous solution of which induced chlorosis and localised water soaking in younger leaves of arecanut. The toxin also wilted cut tomato shoots when dipped in a 0.2 per cent solution and hence is a non-specific toxin. The toxin is a heteropolymer made of glucose, mannose and glucuronic acid.

Keywords : Arecanut, Bacterial leaf stripe, Toxin production, Xanthomonas campestris pv. arecae

Most of the phytopathogenic bacteria are known to produce exopolysaccharides which are phytotoxic and have a role to play in disease syndrome (Rai and Strobel, 1969; Strobel, 1977). Certain of the polysaccharides produced by these plant pathogenic bacteria had wilt inducing properties and were found to be non-specific (Feeder and Ark, 1951). The chemical structure and properties of polysaccharides of some non-pathogens have been studied. Of pathogenic forms, only wilt inducing polysaccharide has been worked out in detail. The exact role of the exopolysaccharide is not clearly known. However, certain of the EPS are known to produce persistent water soaking in host plants (Morris *et al.*, 1977). The present paper reports of the exopolysaccharide toxin of *Xanthomonas campestris* pv *arecae* the causal agent of bacterial leaf stripe of arecanut palm.

MATERIALS AND METHODS : Description and maintenance of bacterial isolate : The isolate *X. c* pv *arecae* purified from diseased leaves of arecanut was grown on the following synthetic media (Leach *et al.*, 1957). Casein hydrolysate 2.0 g, fumaric acid 1.3 g, KH_2PO_4 1.0 g, $MgSO_4$ $7H_2O$ 0 5 g, Na_2CO_3 1.1.g, Fe^{3+} 0.2 g, Mn^{2+} 0.1 g, Zn^{2+} 0.2 g, distilled water 1000 ml, pH adjusted to 7.0. To this basal medium one percent glucose was added. The sterilized medium was inoculated and the shake culture incubated at 28 to 30 C.

Extraction and purification of polysaccharide : The extraction and purification of polysaccharide was carried out following the procedure of Spencer and Gorin (1960). Cultures were grown for 7 days at 28–30°C in a rotary shaker. Bacterial cells were removed by centrifugation at 12000 rpm for 15 minutes. The supernatent was concentrated at 50°C in a flash evaporator to 1/5 of its original valume and treated with 4–5 valumes of acetone and allowed to stand for four hours. The precipitate was filtered and redissolved in distilled water and passed through 20 × 2.5 cm of dowex-50 and then through dowex-1. The effluent from dowex-1 column was collected and dried *in-vacuo*.

Biological assay :The biological assay of the EPS was tested by following the procedure of Hodgson *et al.* (1947). Tomato cuttings (Var. Pusa ruby) of 12"–18"

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were used. Plants of uniform size and leaf area, the upper stem having 2–3 leaves were cut at the collar region and placed in different concentration (0.01, 0.05, 0.1 and 0.2) of aqueous solution of the polysaccharide. One cutting was placed in each concentration. Four replications were maintained and the experiment was repeated thrice. The temperature was maintained at 25–28°C with 70 per cent relative humidity. The time taken for wilting was recorded.

Qualitative estimation of carbohydrate moieties of the exopolysaccharide : An acid hydrolysis of the EPS was carried out by heating on a boiling water bath for 12 hours at 100 to 120°C in 2N H_2SO_4 in a 25×125 mm sealed tube. About 1 ml of acid was used for 5 mg of the exopolysaccharide to be hydrolysed. The acid was neutralised by addition of solid barium carbonate. The solids were removed by low centrifugation or by filtering. The neutralised hydrolysate was concentrated by evaporation *in-vacuo* and further used for qualitative work.

Identification of Sugars : Separation and identification of sugar residues were carried out on silica gel with n-butanol; acetic acid : water (6 : 3 : 1, v/v/v) solvent system. After spraying with the detecting agent (0.5 per cent solution of potassium permanganate in 1N NaOH), the plates were heated to 100°C for 15–20 minutes for colour development and the sugars were detected by their Rf values and by co-chromatography with standards.

RESULTS : *Extraction and purification of exopolysaccharide* : The EPS toxin was isolated from the culture filtrate as indicated earlier. The substance recovered

TABLE 1 : Wilting effect of toxin on tomato cuttings and detached arecanut leaves.

Concentration (g/ml)	Time (hours)				
	0	12	24	48	72
Tomato Cuttings					
0.2	—	—	+++	+++	+++
0.1	—	—	++	+++	+++
0.05	—	—	—	+	++
0.01	—	—	—	—	+
Un-inoculated medium	—	—	—	—	—
Distilled water	—	—	—	—	—
Detached Arecanut Leaves					
0.2	—	—	+	++	+++
0.1	—	—	—	+	++
0.05	—	—	—	—	+
0.01	—	—	—	—	—
Un-inoculated medium	—	—	—	—	—
Distilled water	—	—	—	—	—

—No wilting symptoms; +Slight wilting symptoms exhibited in lower leaves, leaf margins folding inwards, flaccidity, etc., ++ moderate wilting, bottom leaves rolling inwards, slight drooping of upper leaves and growing tip (tomato), flaccidity and dull green colour (arecanut leaves); +++ acute wilting, complete inward folding of all the leaves, growing tip showing complete wilting (tomato), leaves showing wilting and yellowing or chlorosis like symptoms (arecanut leaves).

after passing through the dowex-1 column was referred to as EPS toxin. The yield of toxin was 2 g per litre on dry weight basis.

Biological assay : The studies showed that tomato plants displayed wilting symptoms akin to loss of flaccidity in arecanut leaflets. Initially the test plants under different concentrations of toxin solution lost the turgidity and then started curling of leaves from tip towards the petiole, followed by drooping and wilting. None of these external manifestations could be noticed in controls. At lower concentrations (0.01 per cent) the time required for wilting was more than 72 hours (Table 1).

Qualitative estimation of carbohydrate moieties of the exopolysaccharide : On thin layer chromatography the toxin yielded four chemical compounds viz., glucose, galactose, mannose and glucuronic acid (Table 2). Glucose was in larger proportion than other fractions as observed from the intense colouration of the spot.

TABLE 2 : Composition of the Polysaccharide toxin.

Sugar component	Colour of the spot on spraying	Rf value*
1. Glucose	Brown	0.18
2. Galactose	Brown	0.16
3. Mannose	Brown	0.20
4. Glucuronic acid	Brown	0.14

* Solvent system : n-butanol : Acetic acid. Water (6 : 3 : 1, v/v/v).

DISCUSSION : Plant pathogenic disease causing bacteria have been known to produce phytotoxins. Most of these toxins are non-specific (Rai, 1978; Rai and Strobel, 1969; Strobel, 1977). The mode of action of these toxic compounds has not been fully understood. Majority of the members of *Xanthomonas* are known to produce slime (Gorin and Spencer, 1961). The ability to produce EPS has been related to the virulence of the pathogen (Corey and Starr, 1957). The pathogen *X. c* pv *arecae* also produces a Eps slime which causes wilting, flaccidity and chlorosis in highly susceptible arecanut caltivars. It is an established fact that chlorosis in susceptible host is caused by the EPS produced by the pathogen (Patil *et al.*, 1972).

Wilting in susceptible hosts is due to the formation of the occluding polysaccharide plugs in vessels (Suhayda and Goodman, 1981). The water soluble polysaccharides of high molecular weight induce wilting or may combine with plant cell wall polysaccharide to form a gel similar to the one noticed in *X. c* pv *campestris* (Morris *et al.*, 1977). The occurrence of wilting symptoms in infected arecanut plants may be attributed to occulusion of xylem vessels and the chlorosis part of the disease to the toxic action of the polysaccharide. Sugar moieties have been shown to be responsible for specific water soaking and for toxicity in plants (Strobel, 1977).

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