INACTIVATION OF EXTRACELLULAR POLYGALACTURONASE OF *RHIZOPUS NIGRICANS* EHRENBERG BY PHENOLIC SUBSTANCES

PHENOLIC compounds are known to inhibit or inactivate peetic enzymes (Byrde, 1963)[1]. In the present study, the effect of three phenolic substances, Hydroquinone, Meta-hydroxy-benzaldehyde and Vanillin has been examined on the growth and polygalacturonase activity of *Rhizopus nigricans* in the culture medium. The pathogen was isolated from the rotted papaya fruits.

Papaya broth, the culture medium, was prepared by boiling 250 g of ripe papaya pulp with 300 ml of distilled water for 5 minutes and filtered. The filtrate was made up to a final volume of 500 ml with distilled water, and autoclaved. Cultures were raised in 100 ml flasks containing 25 ml medium with 50,100 or 200 micrograms/ml of the phenolic substances. Inoculum was provided as a 6 mm disc from the periphery of a 3 day old colony growing on potato dextrose agar. Fungus was separated from the medium by filtration and the culture filtrate was directly used as enzyme extract. Cultures without any phenolic substances served as control. Polygalacturonase activity was determined by viscometric procedure (Van Etten *et al.* 1967)[2]. Activity has been expressed as relatiwe enzyme activity (R.E.A.), determined by the formula $1000/t$, where t is the time taken for 25% reduction in the viscosity of the enzyme-substrate reaction mixture, which consisted of 7·5 ml of 1·2% sodium polypectate in water, 3·5 ml enzyme extract, 3 ml glass distilled water and 1·5 ml McIlvains buffer (pH 4·6). Composition of McIlvaines buffer vH 4·6 is, 0·1 M citric acid 106·5 ml and 0·2 M disodiumhydrogen phosphate 93·5 ml.

The results are presented in Table I. The fungus secreted polygalacturonase enzyme into the medium, as revealed by viscosity experiments. Inhibition in the growth of the pathogen was not proportional to the inhibition of polygalacturonase activity, in the presence of phenolic substances. Maximum inactivation was recorded in the presence of vanillin. Results indicate, that tht phenolic substances under consideration, inhibit the polygalacturonase in the medium without interfering much with the growth of the fungus. Polygalacturonase is an important extracellular pathogenic enzyme involved in many diseases. Its inhibition by the phenolic substances of the host may be a major resistant mechanism adapted by the host against the invading pathogen, which advances along with the secretion of polygalacturonase.

TABLE I

Inhibition of extracellular polygalacturonase of Rhizopus nigricans *by phenolic substances*

Phenolic substance	Amount in the medium (micrograms/ml)	Frungal Growth (mg)	% inhibition in growth*	Enzyme activity	% inhibition of enzyme activity*
Hydroquinone	50	905	0·5	55	21·5
	100	903	0·7	45	35·8
	200	902	0·8	40	42·9
Meta-Hydroxy benzaldehyde	50	895	1·6	50	29·6
	100	890	2·2	45	35·8
	200	874	3·9	40	42·9
Vanillin	50	883	1·8	40	42·9
	100	881	2·0	35	50·0
	200	878	3·5	20	71·5

* % inhibition in growth and enzyme activity has been calcuated with reference to the values obtained from control experiments. Each value is the mean of three separate observations, taken after 3 days of fungal growth

The author is grateful to Prof. S. B. Saksena, Prof. of Botany, Saugar University, for his guidance.

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March 4, 1978.

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