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EFFECT OF TRYPSIN ON THE PURIFICATION OF PAPAYA RINGSPOT VIRUS, A *POTY* VIRUS

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The effects of treatment with trypsin during purification of papaya ring spot virus yield and infectivity were examined. These effects were due to reduced aggregation of virus particles at optimum concentration of trypsin whereas at higher concentration of trypsin there were some degradation of virus coat protein rendering loss of virus infectivity. Treatment with trypsin at the optimum concentration can significantly improve purification of papaya ring spot virus.

Keywords: Papaya; Purification; Trypsin; Virus.

Poty viruses comprise, a large and economically important group of plant viruses which infect a wide range of wild and cultivated sp. of plants[1]. They are difficult to purify because they multiply to low concentration in the plant and the particles are prove to aggregate during purification. Papaya ringspot virus was purified by the method given by Purcifull and Hiebert[2], Purcifull *et al*[3] and Gonsalves and Ishii[4]. In an attempt to study the effect of trypsin on virus yield, infectivity of virus and dilution end point, Chenopodium *amaranticolor* an assay host was used. Mean values of local lesions appeared on half leaf was taken into account. Concentration was studiedin triplicate. Results are summarized in Table 1.

It was found that 25 µg/ml trypsin dose is optimum for distinct particle morphology (Fig. 1). On increasing dose of trypsin virus yield is increasing (Table 2) but rate of infectivity is decreasing.

When effect on dilution end point was studied it was found that trypsin raised dilution end point and thus increasing stability of virus preparation.

The results show that poty viruses can become highly aggregated during purification and that this cause low yields and a reduction in the specific infectivity of the product. At low concentration of trypsin digestion of contaminating host proteins and separation of the aggregates into virus particles which leads to higher infectivity, yield and enhancing affects on dilution end point. Trypsin treatment at the higher concentration may cause an additional increase in the degradation of coat protein which is normally caused by plant protease activity. At higher concentration joint action is responsible for the loss of infectivity.

Fig. 1. Clearly visible particles of potato virus Y with the addition of trypsin.

Table 1. Infectivity assay in Trypsin and Non-Trypsin treated preparation.

Experiment	Trypsin (µg/ml)	Lesion/half leaf
1 Set	0	33
	25	147
2 Set	0	20
	25	153
3 Set	0	17
	25	99
4 Set	0	11
	50	28
5 Set	0	29
	50	33
6 Set	0	19
	50	23
7 Set	0	11
	100	3
8 Set	0	27
	100	7
9 Set	0	33
	100	0

Concentration of virus is 8 µg/ml in each set of experiment

Table 2. Effect of Trypsin on virus yield.

Experiment	Trypsin (µg/ml)	Virus yield mg/kg
1 Set	0	1.0
	25	5.2
2 Set	0	2.0
	25	3.9
3 Set	0	1.3
	25	5.42
4 Set	0	0.80
	50	6.0
5 Set	0	0.2
	50	5.34
6 Set	0	1.22
	50	5.23
7 Set	0	1.3
	100	7.2
8 Set	0	7.2
	100	1.29
9 Set	0	1.32
	100	7.27

Samples from single batch, each in triplicate.

Table 3. Effect on dilution end point.

Experiment	With Trypsion (25 µg/ml)	Without Trypsin
1. Set	10^{-5}	10^{-4}
2. Set	10^{-5}	10^{-4}
3. Set	10^{-5}	10^{-4}

(Method followed Noordam, D[5]) each in triplicate.

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