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ENZYMATIC CHANGES IN CARROT ROOTS INDUCED BY ERWINIA CAROTOVORA VAR. CAROTOVORA

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ABSTRACT

Soft rot bacterium causes maceration of tissues due to the activity of various pectolytic enzymes and hence the activity of different pectic enzymes *viz.,* poly galacturanase, pectin methyl esterase, pectin transeliminase and pectate lyase was studied. The activity of all the four enzymes increased from first day after inoculation and decreased on fifth day of inoculation in the carrot roots with all the three bacterial isolates. Among the three, the Coimbatore isolate was found to be more virulent in producing enzymes. The enzyme activity increased from first day after inoculation to fourth day and the activity decreased on the fifth day. Coimbatore isolate was found to produce more of the all pectolytic enzyme studied. When the virulence was compared with the capacity to produce pectolytic enzymes as a tool it could be concluded that the Coimbatore isolate was more virulent among the three isolates tested.

KEY WORDS

Post harvest, Carrot, Soft rot, Erwinia, Enzymes, Diseases.

INTRODUCTION

Carrot *(Daucus carota* L. var. *sativus)* originated from the wild forms growing in Europe and Southwestern Asia. The carrots are grown for their prominent root structure and characteristic flavor and colour. It is valued as food mainly because it is rich source of α and β - carotene. The bacterial soft rot was found to occur universally and its occurrence has been reported from many carrot growing areas. *Erwinia carotovora* subsp. *carotovora* was identified as the causal agent of soft rot. The infected tissues soften, become watery / slimy and as the rot progresses, the watery extrusion becomes evident. A foul odour from the decayed roots distinguishes it from the fungal soft rot. It is observed that the incidence of soft rot is more during rainy seasons at different markets of Coimbatore. There has been very little work on soft rot of carrot and the recent reports are very few. Soft rot bacterium causes maceration of tissues due to the activity of various pectolytic enzymes and hence the activity of different pectic enzymes *viz.,* poly galacturanase and pectin methyl esterase, pectin trans eliminase and pectate lyase were studied.

MATERIALS AND METHODS

The enzymatic changes occurred in the carrot roots at one, two, three, four and five days after inoculation of the pathogen was studied. Uninoculated roots were kept as control.

(i) Polygalacturonase

The activity of polygalacturonase was assayed as per the method described by [1].

One gram of tissue was transferred to a wearing blender and five ml of 0.1M chilled phosphate buffer of pH 6.6 was added. The material was blended for five min. filtered through two layers of cheese cloth and centrifuged at 2000 rpm for 30 min. at 4°C. The supernatant was decanted and the clear extract was taken as enzyme source.

Four ml of the substrate, one ml of acetate buffer of pH 5.2 and two ml of enzyme source were taken in a Vinsell Viscometer of size 300. The contents were mixed gently by drawing air rapidly through the large arm of the Viscometer by suction. The efflux time of the mixture was determined by suction through small arm (Zero time). The efflux time of the mixture after 30 min. was measured. From this, the enzyme activity was calculated as per cent reduction in viscosity of the substrate from the following formula

$$V = \frac{T_0 - T}{T_0 - T_w} \times 100$$

Where

V = Viscosity
T_0 = Flow time in seconds at zero time
T = Flow time of reaction mixture at time T
T_w = Flow time of distilled water

(ii) Pectin methyl esterase

The enzyme activity was assayed as per the procedure given by [1]. Twenty ml of pectin solution was pipetted in a 50 ml beaker and pH was adjusted to 7.0. Ten ml of the enzyme solution was then added and the pH was immediately adjusted to 7.0 by adding 1N NaOH. This was kept as zero time. At every 15 minutes, the pH was checked and alkali was added from the burette when the pH fell below the reference point, while stirring. To adjust the pH, 0.02N NaOH was used and the volume of alkali was noted at each interval. The enzyme activity was expressed as micromoles of hydrogen ion per min/ml (µ mole of hydrogen ion $min^{-1}ml^{-1}$) of the enzyme preparation.

$$PME = \frac{(Vs - Vb)\ NaOH}{Vt} \times 1000$$

where

PME = Pectin Methyl Esterase (μ mole of hydrogen ion $min^{-1}ml^{-1}$)
Vs = Titre value
Vb = Vol. of 1N sodium hydroxide consumed to adjust the pH to 7.0
NaOH = Normality of Sodium hydroxide
V = Volume of incubation mixture (ml)
t = Time period (min)

(iii) Pectin trans eliminase

Pectin trans eliminase activity was estimated by the viscometric method of [1]. Four ml of the substrate and 1 ml of the enzyme were pipetted into the viscometer. The loss in viscosity of the pectin solution was determined by means of Vinsell Viscometer of size 300. The activity was expressed as per cent reduction in viscosity.

$$V = \frac{To - T}{To - Tw} \times 100$$

where

V - Per cent loss in viscosity
T_o - Flow time in seconds at zero time
T - Flow time of reaction mixture at time T
T_w - Flow time of distilled water.

(iv) Pectate Lyase

The estimation of Pectate Lyase activity was done by the procedure mentioned by[2]. The enzyme activity was measured at 25°C by monitoring the increasing absorbance of reaction mixtures at 230 nm. The reaction mixture contained 0.2 ml enzyme sample, 0.8 ml of water and 2.0 ml of 0.1 per cent (w/v) D-galacturonan in 0.2 mM calcium chloride. An increase of one unit of absorbance at 230 nm in this reaction mixture was determined to be equivalent to 3.25 μ moles of unsaturated product per ml of enzyme sample.

RESULTS

Soft rot bacterium causes maceration of tissues due to the activity of various pectolytic enzymes and hence the activity of different pectic enzymes was studied.

(i) Polygalacturonase (PG)

The data from Table 1 showed that the polygalacturonase activity was high in all the inoculated roots than control. The enzyme activity increased from first day after inoculation and decreased on fifth day of inoculation in the roots inoculated with all the isolates. Among the three, the Coimbatore isolate was found to be more virulent in producing enzymes with 96.36 per cent increase over control, which was followed by Ooty isolate with an increase of 69.46 per cent

Table 1

Activity of polygalacturonase in carrot roots induced by E. carotovora** var. **carotovora

Bacterial Isolate	Enzyme activity * (Per cent reduction in viscosity) Days after inoculation					Per cent increase over control	
	1	2	3	4	5	3rd day	5th day
Hosur strain	7.10 (15.45)[b]	11.50 (19.82)[b]	14.96 (22.75)[b]	18.80 (25.69)[b]	15.23 (22.95)[b]	31.11	20.49
Coimbatore strain	12.66 (20.84)[d]	19.16 (25.96)[d]	27.36 (31.54)[d]	31.40 (34.08)[d]	24.82 (29.87)[d]	139.79	96.36
Ooty strain	9.83 (18.27)[c]	14.60 (22.46)[c]	19.56 (26.25)[c]	28.23 (32.09)[c]	21.42 (27.56)[c]	71.43	69.46
Un inoculated control	9.80 (17.46)[a]	10.01 (18.43)[a]	11.41 (19.73)[a]	12.50 (20.70)[a]	12.64 (20.79)[a]		

**** Values are the mean of three replications***
In a column means followed by a common letter are not significantly different from each other at the 5% level by DMRT.
Figures in parentheses are arc sine transformed values

(ii) Pectin methyl esterase (PME)

A progressive increase in the activity of PME was noticed in the inoculated roots of carrot. All the three isolates were found to produce the enzyme. The enzyme activity increased from first day after inoculation to fourth day and the activity decreased on the fifth day of inoculation. The maximum increase in the enzyme activity was observed in the roots inoculated with Coimbatore isolate and Hosur isolate (37.50 per cent) (Table 2).

Table 2

Activity of pectin methyl esterase in carrot roots induced by E. carotovora** var. **carotovora

Bacterial Isolate	Enzyme activity* (Per cent reduction in viscosity) Days after inoculation					Per cent increase over control	
	1	2	3	4	5	3rd day	5th day
Hosur strain	0.08[a]	0.09[bc]	0.11[bc]	0.13[bc]	0.11[c]	57.14	37.50
Coimbatore strain	0.08[a]	0.09[c]	0.11[c]	0.13[c]	0.11[c]	57.14	37.50
Ooty strain	0.08[a]	0.09[b]	0.10[b]	0.12[b]	0.10[b]	42.86	25.00
Un inoculated control	0.07[a]	0.07[a]	0.07[a]	0.08[a]	0.08[a]		

**** Values are the mean of three replications***
In a column means followed by a common letter are not significantly different from each other at the 5% level by DMRT.

(iii) Pectin trans eliminase (PTE)

The inoculated roots showed higher activity of pectin trans eliminase. The enzyme activity increased from first day after inoculation. In contrast to poly galacturanase and pectin methyl esterase, pectin trans eliminase activity was found to show an increasing trend up to fifth day after inoculation. Even though the highest percent increase was noticed on 3rd day after inoculation the highest enzyme activity was noticed on the fifth day after inoculation. The Coimbatore isolate produced more enzyme activity with 167.01 per cent increase over control followed by Hosur isolate with 120.90 per cent (Table 3).

Table 3

Activity of pectin trans eliminase in carrot roots induced by E. carotovora var. carotovora

Bacterial Isolate	Enzyme activity* (Per cent reduction in viscosity) Days after inoculation					Per cent increase over control	
	1	2	3	4	5	3rd day	5th day
Hosur strain	1.06 (5.91)[b]	4.57 (12.34)[b]	11.15 (19.51)[b]	17.66 (24.85)[b]	24.60 (29.73)[b]	134.24	83.58
Coimbatore strain	1.68 (7.44)[c]	6.40 (14.65)[d]	15.61 (23.27)[d]	24.12 (29.41)[d]	35.78 (36.73)[d]	227.94	167.01
Ooty strain	1.59 (7.24)[c]	5.92 (14.08)[c]	14.67 (22.52)[c]	20.24 (26.73)[c]	29.60 (32.96)[c]	208.19	120.90
Un inoculated control	0.00 (0.19)[a]	2.86 (9.73)[a]	4.76 (12.60)[a]	7.80 (16.21)[a]	13.40 (21.47)[a]		

**** Values are the mean of three replications***
In a column means followed by a common letter are not significantly different from each other at the 5% level by DMRT.
Figures in parentheses are arc sine transformed values

(iv)Pectate lyase (PL)

The pectate lyase activity also increased from the first day after inoculation and the increase was consistent up to fifth day after inoculation. The maximum increased enzyme activity of 140 per cent increase was noticed in the roots inoculated with Coimbatore isolate and Ooty isolate (Table 4).

Table 4

Activity of pectate lyase in carrot roots induced by E carotovora** var. **carotovora

Bacterial Isolate	Enzyme activity * (μ moles of unsaturated product/ml) Days after inoculation					Per cent increase over control	
	1	2	3	4	5	3rd day	5th day
Hosur strain	0.005[a]	0.006[b]	0.007[b]	0.009[b]	0.011[b]	40.00	120.00
Coimbatore strain	0.005[a]	0.007[c]	0.008[c]	0.009[c]	0.012[c]	60.00	140.00
Ooty strain	0.005[a]	0.006[b]	0.007[b]	0.009[b]	0.012[c]	40.00	140.00
Un inoculated control	0.005[a]	0.005[a]	0.005[a]	0.005[a]	0.005[a]		

**** Values are the mean of three replications***

In a column means followed by a common letter are not significantly different from each other at the 5% level by DMRT.

DISCUSSION

The degradation of complex polymers of carbohydrates to simple one is brought about by the production of various pectic and pectolylic enzymes produced by *E. carotovora* var. *carotovora*. During post harvest handling and processing, pectolytic microorganisms or cell free pectic enzymes are responsible for tissue maceration[3]. Post harvest rots by nature, suggest maceration due to loss of tissue coherence and separation due to pectolytic activity[4]. The present investigation revealed the production of polygalacturanase, pectin methyl esterase, pectin trans eliminase and pectate lyase in the carrot roots inoculated with all the three isolates of *E. carotovora* var. *carotovora*. It was found that *E. carotovora* subsp. *carotovora* produced pectolytic enzymes which degraded the cell wall[4]. The production of extra cellular endo polygalacturanase and endo pectin trans eliminases by fungi and bacteria was reported by[5]. [6] reported the main distinguishing feature of soft rot *Erwinia* from other *Erwinia* sp. is the ability to produce large quantity of pectic enzymes that enable them to macerate parenchymatous tissues of a wide range of plants.

(i) Poly galacturonase

Increase in poly galacturonase activity was observed in all the three isolates of *E. carotovora* var. *carotovora* tested. This result is in accordance with [7] in carrot roots inoculated with *F. solani* f. sp. *radicicola*. The endo poly galacturonase activity of *Botrytis cinerea* and *Mycocentrospora acerina* in carrot roots was reported by [8].A similar report was also made by [9] in tomato infected with *B. cinerea*. Production of endo poly galacturonase has been correlated with tissue maceration [10].

(ii) Pectin methyl Esterase

A progressive increase in the activity of pectin methyl esterase was noticed from first day to fourth day and start decreasing on the fifth day after inoculation of *E. carotovora* var. *carotovora*. Similar trends were observed in the carrot roots inoculated with *Fusarium solani* f. sp. *radicicola* by [7]. [8] indicated the pectin methyl esterase activity of *B. cinerea* and *M. acerina* in carrot roots. [11] reported the production of pectin methyl

esterase in tomato infected with *Botryodiplodia theobromae* and *Curvularia verucula.*

(iii) Pectin trans eliminase

A consistent increase in the activity of the pectin trans eliminase was noticed in all the three isolates of *E. carotovora* var. *carotovora*. The highest enzyme production recorded on the fifth day after inoculation in the Coimbatore isolate [7] also observed the same trend of increase in the activity of pectin trans eliminase in the carrot roots inoculated with *Fusarium solani* f. sp. *radicicola.*

(iv) Pectate lyase

A steady increase in the pectate lyase activity was reported in the roots treated with all the three isolates of *E. carotovora* var. *carotovora*. The maximum activity was observed in the roots inoculated with Coimbatore isolate. The results are in accordance with [7] in the carrot roots inoculated with *Fusarium solani* f. sp. *radicicola*. *Botrytis cinerea* was found to produce endo pectate lyase when it infected tomato [9]. Increase in the activities of the pectic enzymes may be attributed for the quick decay of the carrot roots under storage. The results are in accordance with [7] in carrot roots inoculated with *F. solani* f. sp. *radicicola*..

The Coimbatore isolate of *E. carotovora* var. *carotovora* was found to produce more of all pectolytic enzymes studied. When the virulence is compared by having the capacity to produce pectolytic enzymes, it could be concluded that the Coimbatore isolate of *E. carotovora* var. *carotovora* is more virulent among the three isolates tested as it produced more enzyme than any others studied. [7] reported that *F. solani* f. sp. *radicicola* was the most virulent pathogen and it produced more pectolytic enzymes. The breakdown of middle lamella by pectolytic enzymes is considered to be an important physiological process after infection [12]. These are in accordance with the present study.

In addition, the changes in the biochemical constituents were also found to be more in the roots treated with the Coimbatore isolate of *E. carotovora* var. *carotovora* indicating the virulent nature of the isolate. [7] also indicated the virulent nature of the *F. solani* f sp. *radicicola* by the ability to change the biochemical constituents. By the above studies, the Coimbatore isolate of *E. carotovora* var. *carotovora* was found to be more virulent.

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