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Biochemical methods for the detection *of Erwinia carotovora varcarotovora* from onion seeds

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Abstract : *Erwinia carotovora* var. *caotovora* causes soft rot of onion bulbs in storage. Three genotypes (N 53, L3V1T2C2, ACSP1) were tested for the presence of soft rot bacterium in seed by roll towel and blotter paper method. A higher per centage of oozing was observed in ACSP1 (81.0 and 83.2 per cent). Studies on morphological character revealed that the causal agent was *E. cartovora* var *carotovora*. The biochemical tests were carried out to identify the causal agents. The bacterium was identified as *Erwinia carotovora* var. *carotovora* and pathogenicity was proved. The seed borne nature of *E. carotovora* var. *carotovora* was proved.

Key words: Onion, *Erwinia carotovora* var *carotovora* and seed.

Introduction

India is the second largest producer of onion in the world followed by China. In India, the area under onion was 3.22 lakh ha with a production of 45.5 lakh tonnes during the year 1996-98 (Anon, 2000). Nearly 40 per cent of the production is lost during postharvest handling and due to sprouting, of which microbial spoilage alone constituted to 15-20 per cent of the total loss (Pantastico and Banstista, 1976; Bhagchandani *et al.* 1980). Soil-borne populations of soft rot Erwiniae have been implicated circumstantially as the primary source of inoculum in storage. The present investigations were aimed at to detect the presence of bacterium in seeds by chemical methods.

Materials and methods

Detection of bacteria from seed

Three different genotypes *viz.* L3V1T2C2, N5 and ACSP1 were collected from the Faculty of Horticulture, Tamil Nadu Agricultural University, Coimbatore. Those genotypes were analysed for the presence of seed-borne bacteria by the standard blotter paper and roll towel methods (ISTA, 1993).

Biochemical methods

(i) Gram staining

Isolates from seeds were smeared on the clean glass slide and allowed to dry. The smear was exposed to flame for two minutes and it was covered with crystal violet for 30 seconds. Then the slide was washed with distilled water for a few seconds and covered with iodine solution for 30 seconds. The iodine solution was washed by 95 per cent ethyl alcohol until no more colour flows from the smear. The slide was again washed with distilled water, drained and safranin (counter-staining) was applied for 30 seconds. The slide was washed with distilled water, blot dried with absorbent paper and air dried. The slide was then examined under microscope using oil immersion objective (Aneja, 1993).

(ii) Starch hydrolysis

The test medium prepared by mixing the starch (15 g) with water (50 ml) until creamy and molten nutrient agar (25 g) was added. The content was autoclaved for 15 min. and dispensed into sterilized Petri dishes. The isolate was streaked on starch agar plates and incubated

Table 1. Detection of seed borne bacteria of onion by blotter paper and roll towel method

Observations	Blotter paper method/ Genotypes*				Roll towel method/ Genotypes*			
	N53	L3V1T2C2	ACSP1	Mean	N53	L3V1T2C2	ACSP1	Mean
No. of seed germinated	11.25[a]	1.25[c]	0.75[c]	4.417	8.00[bc]	4.67[b]	13.33[b]	8.67
No. of seed ungerminated	13.25[a]	23.75[a]	24.50[a]	20.50	14.67[a]	20.00[a]	14.67[b]	16.44
No. of seeds showing bacterial oozing	10.75[a]	18.50[b]	20.25[b]	16.50	9.33[b]	16.67[a]	19.66[a]	15.22
Percentage of oozing	43[c](41.35)	74[b] (60.46)	81[a] (65.16)	66	58.68[c](49.6)	80.00[b](63.7)	83.32[a](66.1)	74.00
Germination percentage	45[a](42.70)	5[b] (13.76)	3[b](12.36)	17.67	32[a](34.9)	18.68[b](24.2)	3.65[c](11.30)	18.11

* Values are the mean of three replications

In a column means followed by a same letter are not significantly different from each other at 5% level by DMRT. Figures in parentheses are arcsine-transformed values

CD (5%) = 4.45

CD (5%) = 2.5

for three days and the plates were flooded with Lugol's iodine solution for colour change and observed (Schaad, 1992).

(iii) Gelatin hydrolysis

The test medium containing beef extract 3 g, peptone 5g, gelatin 120 g, distilled water 1 litre was dispensed in test tube and autoclaved at 121 °C for 12-15 min and cooled until inoculation. After inoculation, the tubes were incubated at 20-22°C for 3 days and kept at 4°C for 30 min and observations on physical state (solid or liquid) were recorded. The gelatin hydrolyzed broth by bacteria turned solid condition at 5°C (Schaad, 1992).

(iv) H_2S production test

The ingredients of test medium *viz.* peptone 30.0 g, beef extract 3.0 g, ferrous ammonium sulphate 0.2 g, sodium thiosulphate 0.025 g, agar 3.0 g distilled water 1000 ml (Schaad, 1992) were dissolved and dispensed in 18 x 150 mm culture tubes and autoclaved at 121°C for 15 min. The test organisms were inoculated on each tube, incubated at 35 -36°C for 48 h and observation on change of colour was made.

(v) Indole production test

The test medium containing tryptone 10 g; L-tryptophan 19 g and distilled water 1000 ml prepared and sterilized. It was dispensed in sterilized Petri dish and test organisms were inoculated. After two days, 0.5 ml of Kovacs' indole reagent was added and the result was observed by change of colour (Aneja, 1993).

(vi) Methyl Red test and Voges Proskauer tests

The MRVP broth containing peptone 7.0 g, dextrose 5.0 g, potassium phosphate 5.0 g, distilled water 1000 ml was prepared, dispensed in test tube and autoclaved at

Table 2. Biochemical tests for the confirmation of *Erwinia carotovora* var. *carotovora*

Sl.No.	Tests conducted	Result	
		Positive	Negative
1.	Gram staining	No	Yes
2.	Sensitivity to erythromycin	No	Yes
3.	Hydrogen sulphide production	Yes	No
4.	Indole production test	No	Yes
5.	Nitrate reduction test	Yes	No
6.	Methyl red test	Yes	No
7.	Caesin hydrolysis test	Yes	No
8.	Starch hydrolysis test	No	Yes
9.	Gelatin hydrolysis test	Yes	No
10.	Sensitivity to penicillin	No	Yes
11.	Growth at 37°C	Yes	No

15 lb pressure for 15 min. The test pathogen was inoculated on two test tubes and one control was maintained and incubated at 35°C for 48 h. Then five drops of methyl red was added and colour change was observed after 5 min.

Twelve drops of V-P reagent-I and 2-3 drops of V-P reagent II were added on another set of test tubes and incubated for 15-30 min. Observation on colour change was made and tabulated (Aneja, 1993).

(vii) Nitrate reduction test

The test medium contained KNO_3 1 g, peptone 5 g, yeast extract 3 g and distilled water 1 litre. The pH was adjusted to 7.0 - 7.2 by addition of 40% NaOH. The medium was dispensed to test tube and autoclaved. The culture of *E. carotovora* var. *carotovora* was inoculated and incubated at 27°C. The tube was examined for evidence of gas production (Schaad, 1992).

(viii) Caesin hydrolysis

Test medium containing skimmed milk powder 100.0 g, peptone 5.0 g, agar 15.0 g, pH 7.2 was prepared and distributed in 250ml conical flasks. Sterilized medium was poured in Petri dishes and single line streak was made on the medium. A control was maintained without streaks and the result on growth of bacteria was recorded (Aneja, 1993).

(ix) Sensitivity to Erythromycin and Penicillin

Nutrient agar medium plus 1% dextrose was prepared and dispensed into tubes and flasks. The tubes and flasks were sterilized at 121°C for 15 min. Medium was poured in Petri dish as basal layer and one drop of nutrient broth was added on the basal layer as seeded medium. The antibiotic disc was placed on the surface of the seed layer and incubated at 27°C for 24-48 h. Observation on growth of the bacteria was made and tabulated (Schaad, 1992).

Results and discussion

Oozing produced by the bacterium from onion seeds and seedling were studied using blotter paper and roll towel methods. Genotypes L3V1T2C2 and ACSP1 showed more pathogen ooze than N52 indicating that L3V1T2C2