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Detection of plasmids in the strains of *Xanathomonas campestris* pv. *cesicatoria* causing bacterial spot of tomato

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SUMMARY
Plasmid profiles were determined for 14 strains of *Xanthomonas campestris* pv. *vesicatoria*, the causal agent of bacterial spot of tomato (*Lycopesicon esculentum* mill) and pepper (*Capsicum annuum* L.). The numbers and size if plasmids in strains from culture collections, as well as in strains recently isolated from plants in commercial field of tomato, were diverse. A single plasmid with a size approximately 30 kb was detected by lysis by boiling method in 9 out go 14 strains.

Key words: Plasmids, tomato, xanathomonas, campestris.

Bacterial spot of tomato (*Lycopersicon esculentum* mill.) and pepper (*Capsicum annuum* L.) caused by *Xanthomonas campestris* pv. *vesicatoria* (Doidge) dye. Low temperature and high rainfall are conductive for disease development. Symptoms initiated as lesions on foliage and fruit, resulting inrapid defoliating leaves and culled fruit under favourable conditions. Control of bacterial spot with copper and/or streptomycin sprays or resistant plants has been erratic because of variation in populations of the bacterium in their resistance to the bactericides and in races that do not interact with resistance genes in the hosts.

Gene for resistance to copper and resistance to streptomycin are plasmid encoded in *X.c.*pv. *vesicatoria* (Bender *et al.,* 1990, and Minsavage *et al.,* 1990). Though the ecological traits in bacteria are plasmid coded, extensive surveys of plasmid content in *X.c.*pv. *vesicatoria* have not been reported. In a preliminary analysis of seven strains of *X.c.*pv. *vesicatoria* two to five plasmids were detected by Dahlbeck *et al.,* (1977).

MATERIALS AND METHODS

Collection of strains:

Fourteen strains of *X.c.*pv. *vesicatoria* were collected from different sources. Two strains from IIHR Bangalore, and one strain from poineer overseas corporation, Bangalore were included. Random survey was conducted to collect different bacterial strains of tomato in Karnataka. Bacterium was isolated from individual lesions, cultured on nutrient agar further purified and stored in refrigerator in sterilized tap water for further use. During active use of the cultures, they were stored in petriplates containing yeast dextrose calcium carbonate agar medium (YDCA) in refrigerator. All the strains were tested for pathogenicity on tomato (Cv. Bonny best) and pepper (Cv. Early calwonder) plants with inoculum of $5x10^6$ cfu/ml. After inoculation, the plants were held on growth chambers at 28ºC. Fifteen plants were maintained for each strains, the cultures were sprayed on to six-eight fully expanded preinjured leaves. Observation were made for the development of symptoms of bacterial spot.

Development of resistance of streptomycin sulfate and copper sulfate:

Fresh stock solution of streptomycin sulphate and copper sulphate prepared in sterile distilled water, filtered at concentrations of 500, 600, 800, 1000, 2000, 4000, 5000, 6000, 7000, 8000 and 10,000 ppm and 0.4, 0.5, 0.7, 0.8, 0.9 and 1.0 percent after passing through 0.22 µm. pore size milli pore filter. Further the concentrations were added to nutrient agar after autoclaving the medium and cooled to 45ºC before planting. Bacterial strains were grown on YDC agar plates for 72 hours and the cells were harvested in sterilized distilled water. The concentration of cell was adjusted to 10^8 cfu/ml. 100 µl of the bacterial suspension was spread on plates containing nutrient agar annmended with streptomycin sulphate and copper sulphate. The plates were incubated at 28ºC for 5 days. One or two colonies developed even at the concentration of 6000 per streptomycin sulphate indicative of resistant strains. In case of copper sulphate. 2-3 resistant colonies were found on medium supplemented with 8000 ppm of copper sulphate.

Isolution of plasmid DNA:

Bacterial strains were grown for 24 hours in tubes containing 2 ml of nutrient both at 30ºC with vigorous shaking, cells were harvested by centrifugation, the plasmid DNA extracted by the method of Holmes and Quigley (1981). Samples were electrophoresed through 0.4% agarose gels in TAE buffer (0.04 M Tris-acetate, 0.001 m EDTA, pH 8.0) stained with Ethidium bromide (2.5 µl/50 ml) for one hr. and photographed using transmitted uv light.

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