Plasmid DNA Isolation from *Xanthomonas campestris* pv. *phaseoli* of Different Geographic Origins Stressed with Cupric Hydroxide

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Plasmid-borne resistance (R-plasmids) to antibiotics and heavy metal compounds is known in plant pathogenic bacteria. The advantages of this type of resistance include multiple-drug resistance that can be carried in a single plasmid and resistance genes that can be amplified when needed and deamplified when not needed. Plasmid DNA has been identified in several pathovars of the bacterium *Xanthomonas campestris* (Canteros, et al., 1995; Fujimoto, 1985; Lazo and Gabriel, 1985). The resistance to copper bactericides by some pathovars of *Xanthomonas* is a problem (Bender, et al.,1990). Self-transmissible copper resistant plasmids have been described in pv. *vesicatoria.* Plasmids of some species are stable and useful for the identification of unknown strains and are important for epidemiological studies. R-plasmid research has important implications in the field of sanitation as well as for the control of bacterial plant disease.

Abstract

A fast minipreparation was used for plasmid isolation and characterization of 20 isolates of *Xanthomonas campestris* pv. *phaseoli* (E.F. Smith) Dowson (Xcp) from different countries in Central America and Puerto Rico. The tested Xcp collection contained strains representing the common and fuscous types. Ninety percent of the Xcp isolates had plasmids of sizes between 1-22 kb, being the most frequent size 15 kb. Xcp fuscans had only one plasmid, while the common types had one to four plasmids. Plasmids found on strains from Nicaragua, Costa Rica, and Puerto Rico were different. Xcp strains from Honduras and Mexico had no plasmids.

Materials and Methods

Xcp was isolated from common blight-infected samples of leaves, pods and seeds collected in Nicaragua, Costa Rica, Guatemala, Honduras, Mexico, and Puerto Rico. The bacterial cultures were purified and tested for pathogenicity on leaves of the common bean, *Phaseolus vulgaris L.,* under greenhouse conditions. Colonies were grown on Luria-Bertani broth amended with 0.1 g/ml of cupric hydroxide. Cultures were activated and used at an optical density of 1-1.5 A. Four ml of each culture were concentrated at 12,000 rpm using a microcentrifuge and washed with 2 ml of distilled water. Covalently-closed circular DNA (ccc-DNA) was extracted using the rapid minipreparation (Series 11453) developed by GIBCO, BRL Products. DNA concentration was estimated using a fluorometer. Plasmids were digested using the restriction enzymes *Bcl* 1, *Csp*45 1, *Eco1*CR 1, *Hae* 11, *Hae* 111, and *Hinf* 1. Agarose gel concentration was 1.0%, and Lamda DNA/*Eco*R 1+*Hind* 111 was used as the marker.

Results

Plasmid ccc-DNA was detected in 18 strains of Xcp representing 90% of the total number of samples tested (20). The sizes of plasmids were between 1to 22 kb, the most frequent being 15 kb. Xcp fuscans had only one plasmid, while those of the common type had between one and four plasmids. One of the strains from Nicaragua had four plasmids and two others, only one. The three strains from Costa Rica had three plasmids; the strain from Guatemala, only one; and the strains from Honduras and Mexico, no plasmids. Over 70% of the strains from Puerto Rico had one plasmid; and the rest two plasmids. Enzymes tested were able to digest most of the plasmids. However, the one that digested the most plasmids was *Bcl* 1.

Conclusion and Discussion

The plasmid profiles resulting from the DNA digestion indicate that the plasmids present in the bacterial strains coming from Nicaragua, Costa Rica, and Puerto Rico are different. This fact supports the potential use of plasmid DNA to characterize strains coming from different countries in Central America and the Caribbean.

Xcp strains collected in Central America represented by the countries of Nicaragua and Costa Rica showed the highest number of plasmid ccc-DNA. Most of the strains from the Caribbean represented by Puerto Rico showed only one plasmid, although 20% of the strains showed two.

The number and sizes of the plasmids detected in this study are less and smaller than those found by Fujimoto. She found strains with up to 5 plasmids and sizes of 1 to 100 kb using non-stressed Xcp strains.

References

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