

## Hormone Production in Xanthomonas campestris pv. phaseoli

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Xanthomonas campestris pv. phaseoli (Xcp) has been differentiated into two phenotypes, CA+ and CA-, in the compatible interaction with the tepary bean, Phaseolus acutifolius. CA+ strains induce water-soaking (WS) with callus proliferation (CA) and CA- induce only WS. Xcp strains representing the CA+, CA- and a non-pathogenic mutant (CA-) were studied in vitro for production of hormones, growth conditions and plasmids profiles; and in planta to determine the role of hormones in pathogenicity or virulence.

### Materials and Methods

Xcp strains used represented different pathogenic races (Zapata, unpublished). A non-pathogenic mutant 827.110 (Arunakumari and Vidaver, 1986), Pseudomonas syringae pv. savastanoi 2009 (T. Gaffney, Univ. of California, Davis), Agrobacterium tumefaciens C 5173 and Escherichia coli were included for comparative purposes.

Production of hormones was determined by the Salkowski reagent, thin layer chromatography, spectrophotometry, gas liquid chromatography (GLC) and monoclonal antibodies. Cultures were grown in a minimal medium (MM) as described by Comai, 1980. The MM was supplemented with L-tryptophan (L-trp)  $\alpha$ -methyl-trp, 5-methyl-trp or DL-trp. Plasmid isolation was performed by following the alkaline lysis procedure described in Maniatis, 1982. Changes in virulence and pathogenicity related to the ability to produce indole acetic acid (IAA) were determined using IAA mutants. Responses to the strains, filtrates and hormones were studied in pods, cotyledons and leaves.

### Results

IAA and indole-3-acetamide were produced by all the Xcp strains when the growth medium was supplemented with L-trp. CA- strains produced higher amounts of IAA than the CA+. The cytokinin isopentenyl adenosin (IPA) and adenine were higher in CA+ than in CA-using a monoclonal antibody and GLC, respectively.

Bacterial filtrates containing IAA did not induce CA, but filtrates in which IAA was not detected induced CA. A differential reaction was induced by IAA in the resistant and susceptible tepary genotypes resembling the reactions with the pathogen. Free trp was found in the seeds of the tepary bean lines. The susceptible genotype showed the highest trp content. CA+ and the non-pathogenic strain but not CA- strains were resistant and grew on media containing trp-analogs. Alterations of the reaction on compatible pod tissues were observed on CA+ strains grown on trp-analogs. On cotyledonary tissues changes in the specificity to the differential host line were observed.

Both CA+ and CA- strains showed two plasmids but some DNA fragments from the CA- differed from the CA+ strains. One of the plasmids of approximately 60kb was not found in the non-pathogenic mutant.

Mutants differing in their ability to produce IAA showed a significantly higher hypersensitivity HR and WS on the incompatible and compatible lines respectively, when inoculated with IAA++ (high producer) versus the IAA- (non producer) and the wild types. On susceptible leaves the CA- induced lesions with progressive chlorosis while the CA+ induced WS without chlorosis.

### Summary and Conclusion

The use of IAA mutants and the non-pathogenic mutant showed that production of IAA is not related as a pathogenicity factor in the interaction of Xcp with the tepary bean. Instead, it is related as a virulence factor. This conclusion is based in the following results:

- a. All strains of Xcp and the non-pathogenic mutant were able to produce IAA when L-trp was added to the growth medium indicating that pathogenicity does not depend on the ability to produce this hormone.
- b. Mutants differing in their ability to produce IAA showed that HR and WS were significantly higher on the incompatible and compatible lines respectively when inoculated with IAA++ versus IAA- and the wild type indicating a role to IAA in virulence.
- c. A differential reaction was induced by IAA in the resistant and susceptible tepary genotypes resembling the reaction with the pathogen. This indicates that IAA can increase the severity of infection on susceptible tissues.
- d. The susceptible genotype showed the highest free trp content as measured by HPLC. This makes feasible the production of IAA by the bacterium.
- e. Ability to produce IAA was associated with CA- strains which induce WS on pods and cotyledons. CA+ were characterized by higher cytokinin content, non-detectable levels of IAA and ability to induce WS and CA on pods and cotyledons.
- f. Ability to produce IAA was associated with the presence of chlorotic margins surrounding lesions on compatible leaves. Non-ability to produce IAA was associated with lesions without chlorotic margins.

### Literature Cited

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