Isolate, identification and real time PCR assay for detection of *Ralstonia solanacearum* Race 3 biovar 2 in asymptomatic potato tubers and other solanaceous crops

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INTRODUCTION

Ralstonia solanacearum is highly destructive and widespread bacterial plant pathogen that is a major limiting factor in the production of many crop plants around the world. The Bacterial wilt (brown rot) disease of potato is among the most serious diseases of potato worldwide and is caused by a subgroup of *R. solanacearum* strains known as race 3 biovar2 (R3bv2) that belong to phylotype IIB sequevar 1 (Fegan and Prior, 2005). Although originally described as strains having a limited host range (Hayward, 1991), R3bv2 strains are not exclusively associated with the potato host and were subsequently reported to also infect tomato, eggplant, geranium, and many weeds and wild plants (Elphinstone, 2005; Swanson *et al*., 2005; Alvarez *et al*., 2008)

World disemination of *R. solanacearum* R3bv2 by potato tubers and geranium cuttings

R3bv2 strains are specifically dangerous:

They can cause symptomless latent infection in seed potato tubers or in geranium

They have a wide host range

They are adapted to highland temperatures and are therefore more cold tolerant than other *R. solanacearum* tropical strains

The potential threat of this bacterium in temperate climates

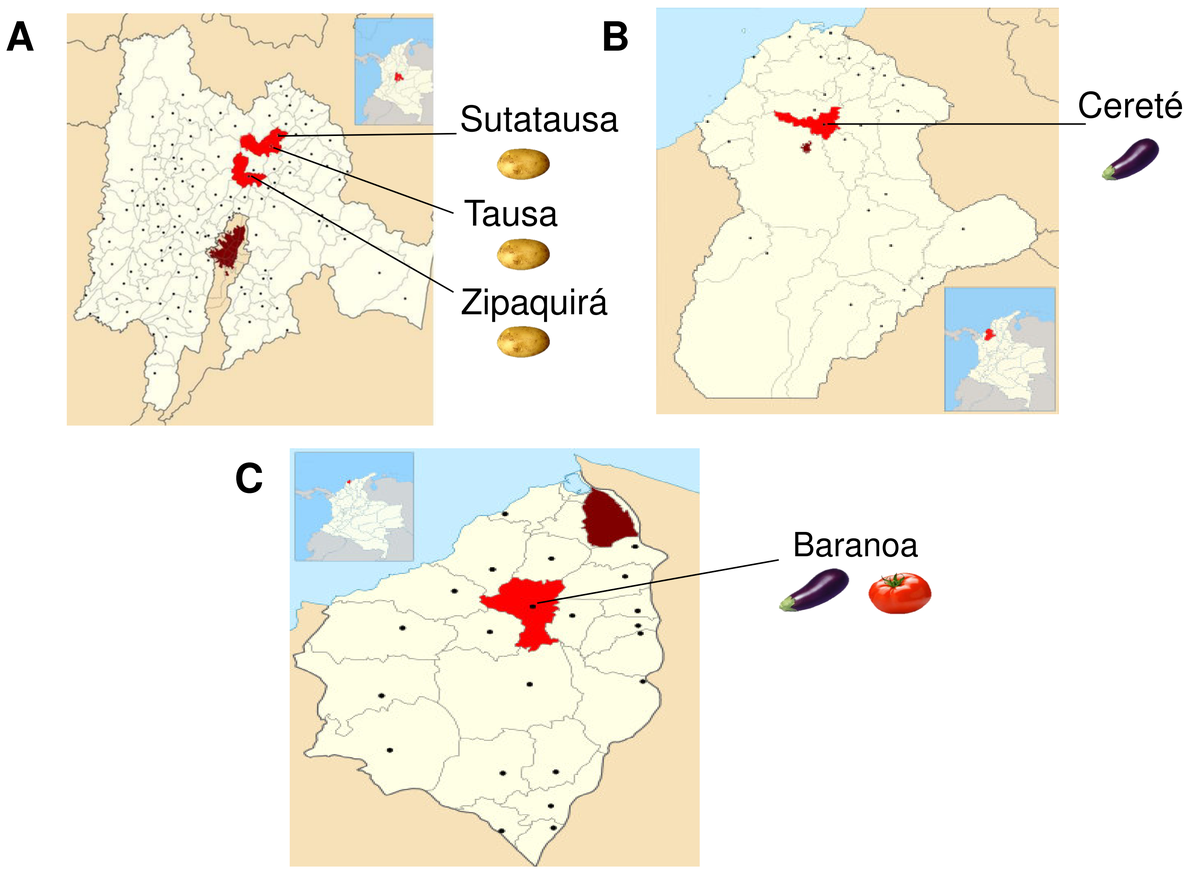
R3bv2 has been classified as a quarantine pathogen in Europe and in North America, but in COLOMBIA is not clear

OBJETIVE

To develop a highly sensitive molecular method for specific detection of strains R3Bv2 of *R. solanacearum* in asymptomatic potato tubers and in other solanaceous hosts.

METHODOLOGY

- ❑ Twenty nine samples composed by potato tubers and plants were collected from potato crops in Cundinamarca
- ❑ Ten samples between eggplant and tomato were collected from Córdoba and Atlántico



Location of the selected municipalities inside Cundinamarca (A), Córdoba (B) and Atlántico (C), Departments of Colombia.

Design of PCR Real Time test

Standardization of Real Time PCR method → Specificity and Sensivity of Primers and Probe → Establishment of the Detection Limit and Ct value

Scheme for detection and identification of *R. solanacearum* in samples of asymptomatic potato tubers and other solanaceous

Pre-treatment and sample Preparation → Process the heel end cores → Concentration of the bacteria → Core Screening Tests (selective isolation, PCR Test) → Detection of *R. solanacearum* R3Bv2 by Real Time PCR → Confirmation by sequencing

RESULTS

- ❑ No plant has shown positive results for strains R3Bv2 until now.
- ❑ Standardization of methodologies for bacteria isolation, DNA extraction and Real Time PCR detection.
- ❑ Generation of documents (Instructions and Analytical Methods) to the Epidemiology and Agricultural Diagnostic Technical Directions from ICA.

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