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 biovar 2 (R3bv2) that belong to phytype 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).

**OBJECTIVE**

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

**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.

**REFERENCES**


**DESIGN OF PCR REAL TIME TEST**

- Standardization of Real Time PCR method
- Specificity and Sensitivity 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
- Confirmation by sequencing
- Detection of *R. solanacearum* R3Bv2 by Real Time PCR
- Core Screening Tests (selective isolation, PCR Test)

**LOCATION OF THE SELECTED MUNICIPALITIES INSIDE CUNDINAMARCA (A), CÓRDOBA (B) AND ATLÁNTICO (C), DEPARTMENTS OF COLOMBIA.**

<table>
<thead>
<tr>
<th>Location</th>
<th>Municipalities</th>
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<tbody>
<tr>
<td>A</td>
<td>Zipaquirá, Sutatausa, Tausa</td>
</tr>
<tr>
<td>B</td>
<td>Cereté, Baranoa</td>
</tr>
<tr>
<td>C</td>
<td>Location of the selected municipalities inside Cundinamarca (A), Córdoba (B) and Atlántico (C), Departments of Colombia.</td>
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