Real-time PCR assays based on the multi-copy rDNA ITS region and the single-copy β-tubulin gene for detection and quantification of the strawberry pathogen *Colletotrichum acutatum*.

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Introduction

*Colletotrichum acutatum* is one of the most important fungal pathogens of strawberry worldwide. In our conditions, disease is mostly expressed as strawberry fruit rot. However, *C. acutatum* may also persist on young strawberry plants without causing visible symptoms. Such latent infections are considered to be the main cause of dissemination of *C. acutatum*. Detection and quantification of *C. acutatum* during this latent phase using real-time PCR might aid considerably in the reduction of its spread.

Objectives

•to develop real-time PCR assays using primers designed to the multi-copy rDNA ITS1 region and the single-copy β-tubulin 2 gene of *C. acutatum*.
•to compare the sensitivity of both assays and to use these data to calculate the genome size and the ITS copy number of *C. acutatum*.
•to detect and quantify *C. acutatum* in infected strawberry leaves.

Methods used

Development and sensitivity of the real-time PCR

The rDNA ITS region and the variable region 1 of the β-tubulin 2 gene of *Colletotrichum* isolates were PCR-amplified, purified and cloned into plasmid vectors. Primers were designed to specifically detect *C. acutatum* (Figure 1). Sensitivity and specificity of the primers was evaluated through amplification and melting curve analysis using SYBRGreen or TaqMan technology. Quantification was done using the standard curve technique.

![Image](https://example.com/image)

**Figure 1.** Partial sequence alignment of the 18S-ITS1 region (A) and the β-tubulin 2 variable region 1 (B), showing the position of the primers (arrows) and probe (line) designed for the detection of *C. acutatum* by real-time PCR. Only polymorphic nucleotides are shown. - indicates a gap in the sequence.

Estimation of the genome size and ITS copy number (based on Lee et al., 2008)

The genome size (GS) of *C. acutatum* was estimated using following equations:

\[X_1 - \left[ \frac{b_3 X_3 + a_3 a_2}{b_4} \right] = 10\]

\[\text{Mass single genome} = \frac{\left[ b_4 X_4 + a_3 a_2 \right]}{b_4} \times 10\]

with 0.996×10⁻²¹ being the average mass of a single base pair and Ct = b₃X₃ + a₃ and Ct = b₄X₄ + a₄ the linear regression curves obtained after simultaneous real-time PCR with gDNA and plasmid DNA of the single-copy β-tubulin 2 gene, resp.

**Results**

Development and sensitivity of the real-time PCR

Using TaqMan technology, the ITS-based assay could reliably detect and quantify as little as 50 fg of gDNA. 100 copies of cloned target DNA, or 25 conidia. The β-tubulin-based assay was circa 66 times less sensitive than the ITS-based assay (Figure 2), and therefore less suitable for detection purposes.

**Figure 2.** Standard curves from SYBRGreen real-time PCR using ITS- & β-tubulin-based primer sets and gDNA from *C. acutatum*.

**Genome size and ITS copy number of *C. acutatum***

We estimated a genome size of 60 Mbp and the presence of circa 20 copies of the ITS region in one genome of *C. acutatum*.

**Detection of *C. acutatum* in inoculated strawberry leaves***

We were able to detect *C. acutatum* in plant tissue mixes of which only 0.001% of the tissue was artificially infected by *C. acutatum*. In addition, the assay allowed monitoring of symptomless growth of *C. acutatum* on strawberry leaves and detection of *C. acutatum* in naturally infected but symptomless strawberry leaves.

**Conclusion**

The real-time PCR assay developed in this study
•is highly specific and sensitive for *C. acutatum*
•proved useful in revealing insights into the genome of *C. acutatum*
•can be used in routine quarantine inspections to screen strawberry plants
•can be used in the study of the biology of *C. acutatum* on strawberry plants

**Reference**


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