Developing diagnostic methods for *Pythium ultimum*

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Introduction

*Pythium ultimum* is a soil-borne plant pathogen causing damping off and root rot diseases in a wide range of crops and ornamental species. In potatoes, Pythium leak is a major storage problem which results in rotten tubers that appear discolored and water soaked (Figures 1 and 2). In addition, infected seed pieces may decay soon after planting resulting in delayed emergence and poor stands. Since similar symptoms in tubers can be caused by other closely related storage rot pathogens such as *Phytophthora erythroseptica* (pink rot) and *P. infestans* (late blight), rapid and robust tests to identify the causal agent would be of great use. Loop mediated isothermal amplification (LAMP) has shown great potential for on site testing, allowing for rapid turn around of results and the timely implementation of management decisions.

The objective of this study was to develop a rapid and robust LAMP assay specific for *Pythium ultimum* and compare it with existing laboratory based assays.

Materials and Methods

- DNA was extracted from cultures, soil and tubers using a Wizard Food Kit (Promega) in conjunction with a Kingfisher ML magnetic particle processor (Thermo Scientific UK Ltd).
- A LAMP assay with putative specificity to *Pythium ultimum* var. *ultimum* was designed to the rDNA ITS region.
- Specificity was evaluated against 15 closely related *Pythium* species and other potato pathogens including *Rhizoctonia*, *Phytophthora*, *Spongospora* and *Alternaria* species.
- Sensitivity of the LAMP assays was compared against previously published TaqMan (Cullen et al., 2007) and SYBR Green (Schroeder et al., 2006) real-time PCR assays with serial dilutions of pure culture DNA and also with DNA from tuber and soil samples.
- LAMP assays were undertaken using isothermal Mastermix and a Genie® II instrument (Fig. 3). A total volume of 25 µl was used for each LAMP reaction which was run for 40 minutes followed by an anneal step from 90 to 80°C.
- Real-time PCR assays were undertaken with Environmental Master Mix 2.0 and PowerUp SYBR Green master mix (Thermo Fisher).
- Serial dilutions of humic acid were used to determine the level of tolerance each assay had to the inhibitor.

Results and Conclusions

- The LAMP assay was specific to *Pythium ultimum* var. *ultimum* and did not detect other species.
- The limit of detection of the LAMP assay was greater than the SYBR green assay but ten times less than the TaqMan assay (Figs. 4 & 5).
- The LAMP assay was highly efficient (Table 1) with time to positive values between 5 and 21 minutes (Figure 5 & Table 2).
- However, the LAMP assay was less tolerant of humic acid, a commonly found inhibitor in soil (Table 1).
- This may explain why less soil samples tested positive with the LAMP assay compared to the TaqMan assay. No soil or tuber samples tested positive with the SYBR Green assay.
- The LAMP assay may provide a useful diagnostic assay to rapidly identify cultures and detect the pathogen tuber material.
- Further work will focus on integrating the LAMP assay into an onsite test for tuber disease in conjunction with rapid DNA extraction methods.

Table 1. Limit of detection, reaction efficiency and humic acid tolerance level for each assay.

<table>
<thead>
<tr>
<th>Assay type</th>
<th>Limit of detection (fg)</th>
<th>Reaction efficiency (%)</th>
<th>Level of tolerance to humic acid (ng)</th>
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<tbody>
<tr>
<td>TaqMan</td>
<td>2</td>
<td>92</td>
<td>28</td>
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<td>SYBR-green</td>
<td>200</td>
<td>70</td>
<td>10</td>
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<td>LAMP</td>
<td>20</td>
<td>783</td>
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References