CHARACTERIZATION OF MICROORGANISMS ASSOCIATED WITH POTATO TUBER ROTS

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Background: Potato tuber rots are one of the major causes of post-harvest losses worldwide and are commonly associated with both fungal and bacterial pathogens. Dry rot is primarily caused by several species of the genus *Fusarium*, whereas soft rot is mainly associated with *Pectobacterium* and *Dickeya* genera. Infected tubers can contribute to the persistence and spread of these pathogens in storage facilities and agricultural systems. A comprehensive understanding of the diversity of microorganisms associated with potato tuber rot is essential for the development of effective disease management strategies.
The aim of the present study was to isolate and identify microorganisms associated with dry rot and soft rot of potato tubers from the active collection of local potato germplasm maintained at the National Gene Bank of the Institute of Genetics, Physiology and Plant Protection.
Materials and methods: Tubers from ten potato genotypes exhibiting symptoms of different types of rot were analyzed. A total of eighteen microbial isolates were obtained in pure culture from infected potato tissues displaying symptoms of dry rot and soft rot. Fresh fungal mycelium or bacterial biomass grown on culture media was collected after five days of incubation and subjected to DNA extraction using a modified CTAB protocol. The concentration and quality of the extracted DNA were assessed by spectrophotometric analysis and agarose gel electrophoresis. Polymerase chain reaction (PCR) was employed for the molecular identification of the isolates. Fungal isolates were amplified using universal ITS primers targeting the internal transcribed spacer (ITS) region of ribosomal DNA, whereas bacterial isolates were analyzed using primers specific to the 16S rRNA gene. PCR reactions were carried out in a total volume of 25 µL containing 66 mM Tris-HCl (pH 8.4), 16 mM $(NH_4)_2SO_4$, 2.5 mM $MgCl_2$, 0.1% Tween 20, 7% glycerol, 100 µg mL^{-1} bovine serum albumin, 0.2 mM of each dNTP, 1 U Taq DNA polymerase, 5 pM of each primer, 50 ng of genomic DNA. Amplification was performed under the following conditions: initial denaturation at 95 °C for 4 min, followed by 35–40 cycles of denaturation at 95 °C for 30 s, primer annealing at 55–60 °C for 30 s (depending on the primer set), and extension at 72 °C for 45–60 s, with a final extension at 72 °C for 10 min.
Results: The results showed that twelve isolates produced positive amplification with ITS primers, indicating their fungal origin, whereas six isolates yielded positive amplification with 16S rRNA primers, suggesting a bacterial identity. Subsequent PCR analysis of the ITS-positive isolates using β-tubulin gene primers confirmed the presence of *Fusarium* spp.
Conclusions: These findings contribute to a better understanding of the microbial complex associated with potato tuber rot and provide a basis for further molecular identification and characterization of the pathogens involved.
Keywords: potato germplasm, dry rot, soft rot, PCR.
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