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Fourier Transform Infrared (FTIR) Spectroscopy Method for *Fusarium solani* Characterization

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KEYWORDS

Carbohydrate;
FTIR spectroscopy;
Fusarium solani;
PCR;
Wavenumber.

Abstract The detection and identification of microorganisms using spectroscopy techniques promise to be of great value because of their sensitivity, rapidity, low expense, and simplicity. In this study, we used FTIR spectroscopy for the characterization of *Fusarium solani.* PCR amplification of DNA extracted from these isolates showed the possibility of amplifying PCR products with sizes 559 bp using the ITS1-ITS4 primers. Based on phylogenetic tree analysis, the isolate of *F. solani* showed a closely relationship to *Fusarium solani* isolate MN (MH300495.1) with 99.63% similarity. The study is focused on the carbohydrate structure which can be analyzed in the range of 900 to 1200 cm^{-1} of FTIR wavenumber. The spectra of our samples share similarities with one another, although small differences occur in the absorbance value. The band at 1027 cm^{-1} is assigned to the C-O stretching of glycogen. Meanwhile, at 1042 cm^{-1} is interpreted as carbohydrate C-O stretching as well. The band around 1073 cm^{-1} might arise from both chitin C-C stretching and phosphate stretching of nucleic acids. Other vibrations associated with chitin are also found at 1115 cm^{-1} and 1151 cm^{-1} which are assigned to C-O-C symmetric stretching and C-O-C asymmetric stretching, respectively.

Introduction

Fungal pathogens cause serious damage to many crops with significant negative feedback on the economy. Early identification enables precise targeting of a pathogen and enables the most effective treatment. Most commercially available identification systems for fungi are based on physiological characteristics. Such identification systems are usually time-consuming and not always very specific. Among the techniques offering possibilities for rapid analysis, molecular biology methods are considered the most rapid and sensitive methods for the identification of pathogens, but they are not yet in large-scale use.

The detection and identification of microorganisms using spectroscopy techniques promise to be of great value because of its sensitivity, rapidity, low expense, and simplicity. FTIR spectroscopy is an attractive technique for the detection and identification of pathogens. The principle of FTIR spectroscopy is to detect chemical compounds from the infrared spectrum produced to provide a unique and specific "fingerprint" (Salman *et al.*, 2010). Several studies on the use of FTIR spectroscopy are the detection and characterization of cancer cells, cells infected with viruses and microorganisms, including some fungi; early detection of fungal infection *Colletotrichum coccodes* on potato tubers (Erukhimovitch *et al.*,

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2007). Detection and identification of four different fungal genera that infect potato tubers (Erukhimovitch *et al*., 2010). Identification of the fungus *Pythium* spp. and *Fusarium* spp. (Erukhimovitch *et al*., 2005). Detection of fungus on wood (Naumann *et al*., 2005).

In the present study, we used FTIR spectroscopy for the characterization of *Fusarium solani*, potato fungal pathogens.

Materials and Methods

Sampling and Fungal Isolation

Potato tuber samples were collected from diseased potatoes in the storage in Sumberbrantas-Batu, East Java. The samples were transferred to the laboratory of mycology at the "Agricultural Quarantine Major Service of Surabaya" for the isolation of fungal pathogens. Collected tubers were washed with tap water, cut into small pieces (1x1 cm), sterilized with NaOCl (1%) solution for 2 min, and washed with sterile distilled water to remove any residues of NaOCl. Tuber pieces were then dried using filter papers to remove any excess water and transferred to Petri dishes containing 3 sheets of wet filter paper, incubated at a temperature of 20-24°C for about 7 days. The appeared fungi were purified with potato dextrose agar (PDA). Supplemented with chloramphenicol antibiotic at a concentration of 100 mg/L (Samson *et al.*, 1995).

Morphological Identification

The appeared fungi were purified and maintained on the PDA and were used for morphological identification by microscopic examination.

Molecular Characterization

The isolated fungi were molecularly identified using PCR techniques and determining the nucleotide sequence. Molecular characterization of *F. solani* isolate was described in Figure 1.

*DNA extraction***.**

Fungi isolate, 50-100 mg of fresh 7-days-old colonies were taken by a sterile scalpel and transferred into an Eppendorf tube for DNA extraction using a specific extraction kit Zymo

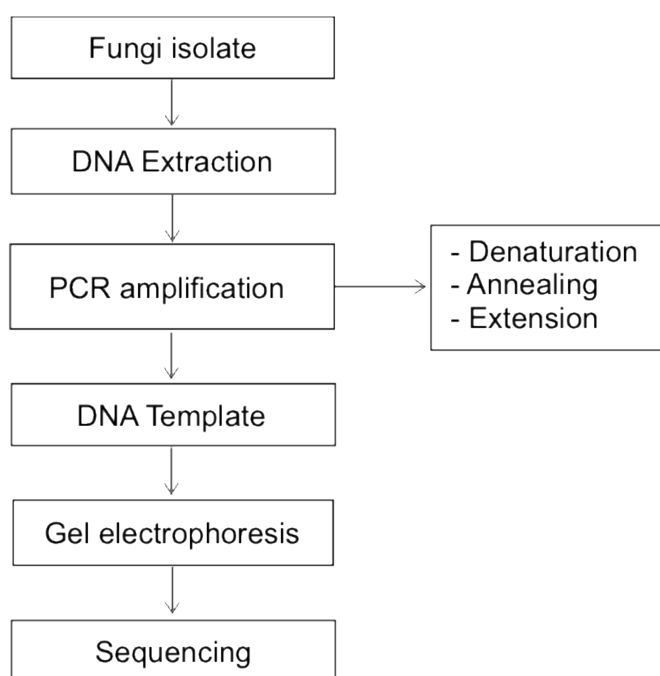

Figure 1. Molecular characterization of *F. solani* isolate

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Quick-DNA Fungal/Bacterial Miniprep Kit (Zymo Research, Cat. No. D6005), following the manufacturer's instructions. DNA was then stored at -20 until use.

*PCR amplification***.** The internal transcribed spacer (ITS) region of all fungi isolates was amplified, using the universal primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (Singha *et al.*, 2016) using MyTaq HS Redmix (Bioline, 25048). The final volume of each PCR reaction mixture (sample) was 25 μl containing: 12.5 μl MyTaq Redmix, 1 μl (10 μM ITS1 Primer), 1 μl (10 μM ITS4 Primer), 1 μl template DNA (30 ng/μl), then completed to 9.5 μl by adding ddH_2O. PCR amplification was performed using the following conditions: initial denaturation at 95°C for 3 min followed by 35 cycles each consisting of final denaturation at 95°C for 10 s, annealing temperature at 52°C for 30 s, and final extension at 72°C for 45. PCR-amplified products were electrophoretically separated on a 1% TBE agarose gel.

DNA sequencing. The fungi target DNA that was successfully amplified was then sent to Apical Scientific Sdn Bhd, Malaysia. The nucleotide sequences for each isolate were then compared to those in the public domain databases NCBI (National Center for Biotechnology Information; www.ncbi.nih.gov) using the Basic Local Alignment Search Tool for Nucleotide Sequences (BLASTN). Alignment of ITS DNA sequences was done using the UPGMA method program. Using Mega11 software, multiple alignments of nucleotide sequences and the construction of phylogenetic trees were performed using the neighbor-joining method (Tamura *et al.* 2021). The confidence of the branching was estimated by bootstrap analysis with the value of 1000 times.

Sample preparation

Based on the methods of Erukhimovitch *et al.* (2010). Small samples of the fungal pathogen were obtained from infected areas of potato tubers and grown in appropriate culture agar. Samples of these fungi were purified from the media by spinning about 1 ml of medium containing fungi at 2000 rpm for 5 min and washing twice with H_2O, after which the pellet was suspended in an appropriate volume of H_2O (about 50 μl). A drop of 10 μl of the obtained suspension was placed on a certain area on the zinc selenide crystal, air dried, and examined by FTIR spectroscopy.

Fourier Transform Infrared Spectra Measurement

The samples obtained were analyzed by the ATR method at wave numbers of 400-4000 cm^{-1}. All samples were in liquid/suspension. Each sample was taken at 47 mg. Then, the FTIR spectrophotometer (JASCO 6800) was blank with air as the sample measurement background. The sample was then measured for its FTIR spectrum with an FTIR spectrophotometer with a TGS detector and attenuated total reflectance (ATR, Pro One) at an incident angle of 45 deg and a scanning speed of 2 mm/second.

Results and Discussion

Characterization of Fusarium solani

From the results of this study, it is known that *Fusarium solani*, cultures on PDA had white colonies on the top surface of the Petri and yellowish white on the bottom of the Petri. *F. solani* isolate colonies were able to fill a 9 cm diameter petri dish after 14 days of incubation. Hyaline conidiophores are simple and there is a mass of spores at the top. Hyaline conidia are of two types: macroconidia with slightly curved apical cells, often slightly curved on one side, and bent leg cells, usually 3 to 5 cells. Cylindrical microconidia, 1-2 cells. Conidia: macroconidia have 26.76 µm and microconidia have 10.79 µm. Morphological characteristics was described in Figure 2.

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For confirmation, the morphological identification of *F. solani* (1=sample code, M=Marker 100 bp). PCR amplification of DNA extracted from these isolates showed the possibility of amplifying PCR products with sizes 559 bp using the ITS1-ITS4 primers (Figure 3). Based on phylogenetic tree analysis, isolate *F. solani* showed closely relate to *Fusarium solani* isolate MN (MH300495.1) with a percentage identity of 99.63% (Figure 4).

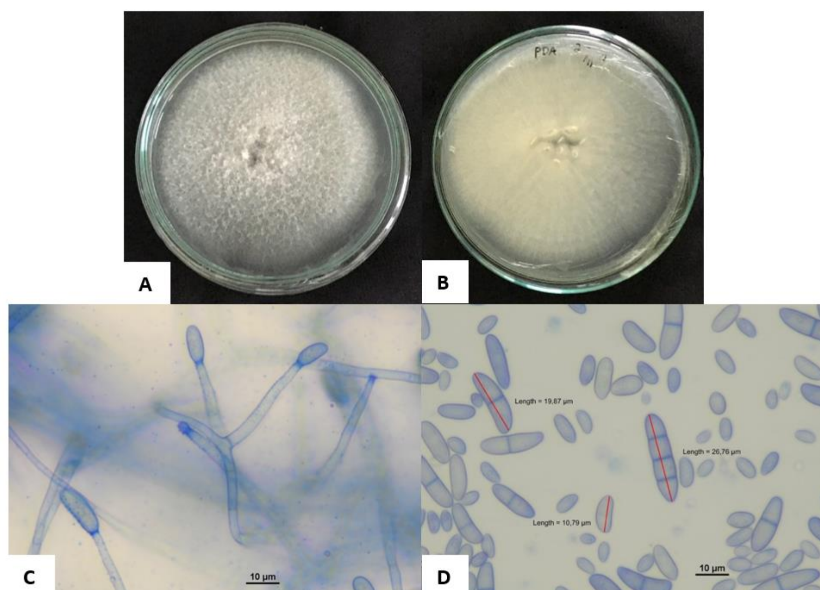

Figure 2. Morphological characteristics of *F. solani* (A-B) aerial view and reverse, (C) Conidiphores, (D) Macroconidia and microconidia structures, bar=10 µm.

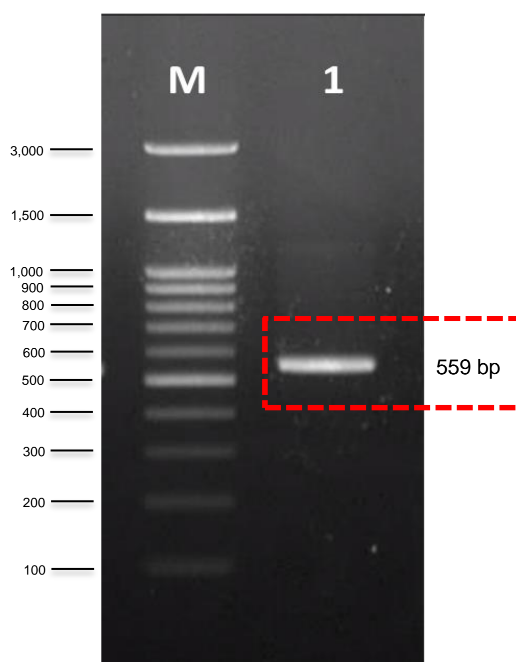

Figure 3. DNA products amplified by polymerase chain reaction (PCR) of *Fusarium solani*, M=100 bp DNA ladder (loaded 2.5 µl). (M=marker; 1 = *Fusarium solani* fungi/sample code)

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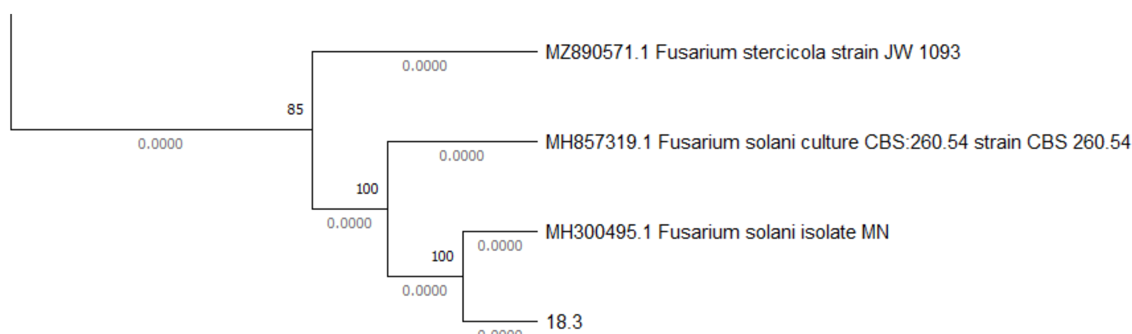

Figure 4. A phylogenetic tree was generated using the neighbor-joining method showing the genetic relationship between the *Fusarium solani* isolate (18.3) and other *F. solani* isolates (Mega 11 software).

FTIR spectra of Fusarium solani

Samples obtained from *F. solani* isolate were examined by FTIR spectroscopy to find specific spectroscopic biomarkers for rapid identification and discrimination. Developing specific biomarkers by FTIR spectroscopy could be highly important for future rapid and reliable detection and identification of these pathogens.

The study is focused on the carbohydrate region which spans from 900 to 1200 cm^{-1} wavenumber (Figure 5). Each set of spectra shares similarities one to another, although differences are still occurred. Some bands associated with carbohydrates are found in the fungal isolate. The band at 1027 cm^{-1} is assigned to C-O stretching of glycogen (Salman *et al*., 2012). Meanwhile, at 1042 cm^{-1} is interpreted as carbohydrate C-O stretching as well (Salman *et al*., 2010). The band around 1073 cm^{-1} might arise from both chitin C-C stretching and phosphate stretching of nucleic acids (Salman *et al*., 2012). Other vibrations associated with chitin are also found at 1115 cm^{-1} and 1151 cm^{-1} which are assigned to C-O-C symmetric stretching and C-O-C asymmetric stretching, respectively (Waśko *et al*., 2016).

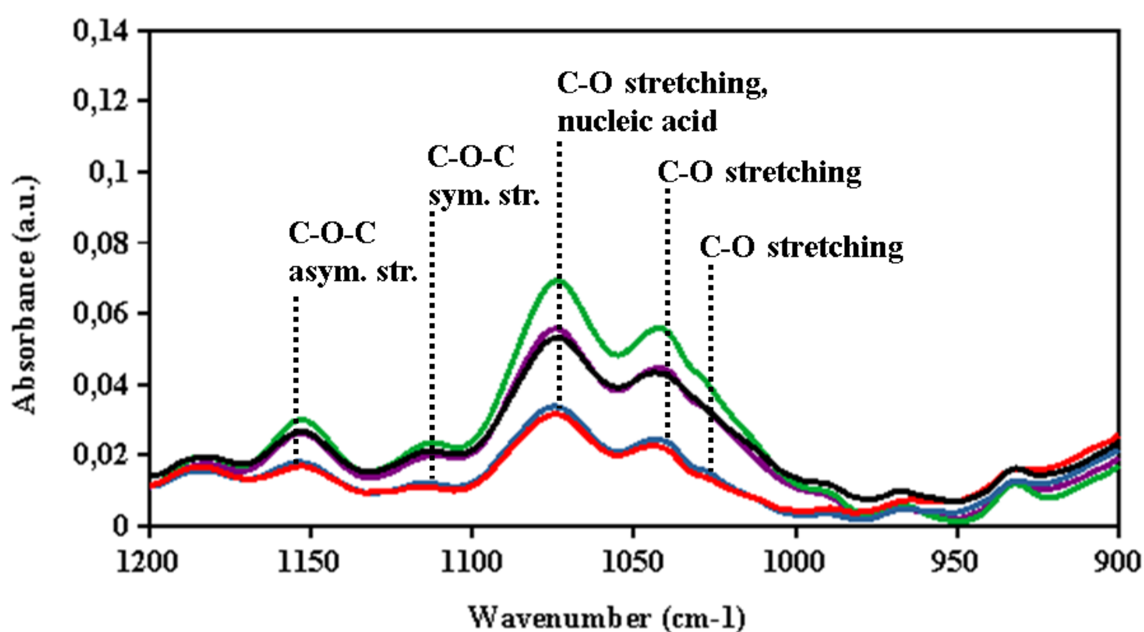

Figure 5. ATR-FTIR absorbance spectra of *Fusarium solani* samples

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Conclusions and Suggestion

In this study, we examined the potential of FTIR spectroscopy as rapid characterization method of *F. solani*. The study is focused on the carbohydrate structure which is observed in the 900 cm^{-1} to 1200 cm^{-1} region of FTIR spectrum. We discover a unique and consistent spectral marker of *F. solani*. The spectra of our samples share-obvious similar pattern on to with small differences in the absorbance value. Some bands associated with carbohydrates are found in the fungal isolate.

Acknowledgments

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