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Fourier Transform Infra-Red (FTIR) Spectrum Characterization of *Bacillus Mycoides*

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KEYWORDS
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Spectrum;
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Abstract The presence of *Bacillus mycoides* and its ability to grow and spread quickly certainly affect the growth of the target pathogen and it can cause invalid detection results. Therefore, the presence of contaminant bacteria needs to be detected to ensure the specificity of the detection results against the target pathogenic bacteria. Various kinds of detection methods are commonly used, such as ELISA (*enzyme-linked immunosorbent assay*) and PCR (*polymerase chain reaction*) are time-consuming and not always very specific. Fourier-transform infrared (FTIR) spectroscopy methods were adopted to provide a comprehensive and reliable method for bacterial analysis. In this study, FTIR spectroscopy was used as an initial guess for the identification of bacterial isolates. Our results showed that there are dominant peaks from the FTIR spectrum obtained that were most associated with protein and carbohydrate in the range of wave number 400-4000 cm^{-1}.

Introduction

In the effort to detect pathogens that cause disease in plants, the presence of other pathogens and saprophytic organisms affects the specificity of the detection results. *Bacillus mycoides* is one of the saprophytic bacteria in melon seeds (Zhao et al., 2009). The presence of *B. mycoides* and its ability to grow and spread quickly certainly affect the growth of the target pathogen and it can cause invalid detection results. Therefore, the presence of contaminant bacteria needs to be detected to ensure the specificity of the detection results against the target pathogenic bacteria.

Various kinds of detection methods are commonly used, such as ELISA (*Enzyme-Linked Immunosorbent Assay*) and PCR (*Polymerase Chain Reaction*),. But generally,t takes a long time and has high costs. It is very important to recognize the specific chemical structure of a particular species. The chemical structure of a species can be identified using the Fourier-transform infrared (FTIR) spectroscopy method. FTIR spectroscopy is a reliable, fast, and economic technique which can be explored as an ordinary diagnostic tool for bacterial analysis (Davis & Mauer, 2010). In this study, the FTIR spectrum of *B. mycoides* isolate obtain was characterized for the identification of bacterial isolates.

Materials and Methods

Bacteria Sample

The bacterial isolates used in this study came from the HPT UB collection by Dinata et al. (2021). Bacterial isolates from stocks were grown on Nutrient Agar (NA) for 24-48 hours at room temperature to ensure purity.

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Molecular Identification

The pure isolates obtained were then identified. Molecular identification of pure isolates was carried out using the PCR method.

DNA extraction

Bacterial isolates aged 24-48 hours on NA growth medium were taken as much as 1 loop and dissolved in 1 ml of sterile water in a 1.5 ml tube. The bacterial suspension was centrifuged at 10,000 rpm for 10 minutes, the pellet was used for DNA extraction.

The DNA extraction procedure follows the instructions of DNeasy Blood and Tissue Kit, Qiagen. The extracted DNA is used as a DNA template in the PCR process.

PCR Amplification and Visualization

PCR testing for *B. mycoides* bacteria using universal primer 16S (27F/1492R) with the following primer base arrangement Heuer et al., (1997): 27F (5'-AGAGTTTGATCCTGGTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-3'). The PCR components used were as follows: 12.5 µl Dream Taq HS Green PCR Master Mix (2X), 1 µl Primer 27F (10 µM), 1 µl Primer 1492R (10 µM), 1 µl Template DNA, and 9.5 µl Nuclease Free Water so that the total volume is up to 25 µl.

PCR amplification was carried out under the following conditions: 1 cycle of Predenaturation at 95°C for 5 minutes, 35 cycles for Denaturation at 95°C, 30 seconds; Annealing at 42°C, 30 seconds; Extension at 72°C, 30 seconds, 1 cycle of Final Extension at 72°C, 5 minutes.

The electrophoretic process of the PCR-resulted DNA was carried out using 0.8% agarose gel with Gel Stain (Smobio) in TAE Buffer at 70 volts for 60 minutes. Visualization of PCR results using the Vilber Lourmat Gel Documentation System.

DNA Sequencing

The amplified target bacterial DNA was then analyzed by PT. Genetics Science Indonesia whose results were forwarded for phylogenetic analysis. The DNA sequences of the isolates obtained were analyzed phylogenetically together with isolates from several other countries so that their genetic relationship could be identified. Phylogenetic analysis using Molecular Evolutionary Genetics Analysis (MEGA 11) software and Basic Local Alignment Search Tool for Nucleotide Sequences (BLASTN) from the National Center for Biotechnology Information (NCBI)(Tamura et al., 2021).

Characterization of Sample FTIR Spectrum
Sample preparation

Bacterial samples that have been identified molecularly by the PCR method are then tested for their FTIR spectra Erukhimovitch et al. (2005) with a few modifications. Bacterial isolates were grown in Nutrient Agar (NA) medium for 24-48 hours at room temperature. Small amounts of bacterial colonies were collected and suspended in 1 ml of sterile distilled water. The suspension was centrifuged at 10,000 rpm for 10 minutes. The pellet was washed twice with sterile distilled water. The pellets were suspended in 50µl sterile distilled water. The suspension obtained is then taken 30µl and placed in a certain area of zinc selenide crystal, dried, and tested using the FTIR spectroscopy method.

Fourier Transform Infrared Spectrum Measurement

The sample FTIR spectrum was measured using an FTIR spectrophotometer (JASCO 6800) 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. Measurements were made in the wave number range of 400-4000 cm-1. The sample spectra were collected after the background spectra were taken. Spectral testing was carried out with 5 repetitions.

Data from the FTIR test results obtained from the spectra manager software (in CSV

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format) are converted into excel format so that the spectra pattern can be processed and visualized in graphical form. From the graph obtained, the average peak value in the sample spectra pattern is displayed so that the peak position of the sample spectra can be known at a certain wave number.

Results and discussion

Bacterial Identity

Based on the results of the purification of *B. mycoides* bacterial isolates on NA growth media, it can be seen that the morphology of *B. mycoides* bacteria colonies is shown in Figure 1. Bacterial colony morphology is rhizoidal and milky white. According to Andriani et al. (2017), *B. mycoides* looks white, and coarser with fine threads around the colony, the color is milk white, and the cells are rod-shaped, gram-positive, and sporous.

Molecular identification of *B. mycoides* was carried out by the PCR method using universal primer 16S (27F/1492R). The results of *B. mycoides* bacterial DNA amplification is shown in Figure 2. Based on the results of PCR visualization (Figure 2) it is known that the DNA band of the tested *B. mycoides* isolate was amplified at 1426 bp. The amplification of the sample at this size indicated that the universal primer 16S (27F/1492R) used in PCR was able to detect the DNA sample.

The results of the DNA sequence analysis of PCR products using BLASTN are shown in Table 1. The data in Table 1 shows the level of similarity of the tested *B. mycoides* isolates samples compared to isolates from several other countries in the NCBI database. Based on the data in table 1, it is known that the tested *B. mycoides* isolate sample has similarities with the *B. mycoides* strain KUDC1724 sequence from South Korea with accession number KC414703.1, *B. mycoides* strain ARD19 from India with accession number KX023234.1 and *B. mycoides* strain TF3-30 from China with accession number KJ127234.1. Each sequence compared to the test sample has a similarity level of 100%.

The phylogenetic relationship between the tested *B. mycoides* isolates and other isolates from several countries is visualized in the form of a phylogenetic tree using the software MEGA 11 as in Figure 3. Based on the results of the phylogenetic tree construction, it can be seen that the tested *B. mycoides* isolate samples are closely related to the *B. mycoides* strain KUDC1724 isolate from South Korea with accession number KC414703.1 with a 100% similarity level.

FTIR Spectrum Characterization

In line with the results of molecular identification of bacterial samples, the tested *B. mycoides* bacterial isolates had similar FTIR spectral patterns among the replicates (Figure 4). This indicates that testing with FTIR spectroscopy is stable and reliable (Wang et al., 2012). The chemical characteristics of the sample can be described in the form of an FTIR fingerprint spectrum which is very complex data information(Tarapoulouzi et al., 2020).

Characterization of the FTIR spectra of the tested bacterial isolates was carried out based on the average FTIR spectra of the bacterial isolates obtained (Figure 4). The average spectrum at wave numbers 400-4000 cm^{-1} shows that the dominant peaks are at 1190 cm^{-1}, 1419 cm^{-1}, 1474 cm^{-1}, 1591 cm^{-1}, 1715 cm^{-1}, 3305 cm^{-1} and 3365 cm^{-1}.The peak at 1190 cm^{-1} is attributed to C–OH, C–O–C, and C–O ring stretching of carbohydrates (Grunert et al., 2018; Lasch & Naumann, 2015). The peak at 1419 cm^{-1} was assigned to COH in plane bending of carbohydrates (Davis & Mauer, 2010). The peak at 1474 cm^{-1} represents C–H deformation of >CH2 of protein-lipid (Davis & Mauer, 2010). The peak at 1591 cm^{-1} is assigned to NH2 stretching, C=O, and C=N stretching of protein (Maity et al., 2013). The peak at 1715 cm^{-1} is attributed to the C=O esters stretching of RNA/

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DNA (Lasch & Naumann, 2015). The peak at 3305 cm^{-1} was assigned to N–H stretching (amide A) of proteins (Lasch & Naumann, 2015). The peak at 3365 cm^{-1} represents O–H and N–H stretching vibrations of hydroxyl groups and amide A of proteins (Bombalska et al., 2011). The most main peaks of the spectral pattern of *B. mycoides* isolates are associated with proteins and carbohydrates. According to Andriani et al. (2017), *B. mycoides* is a non-motile bacterium that can extract acid from glucose, hydrolyze starch and produce protease enzymes.

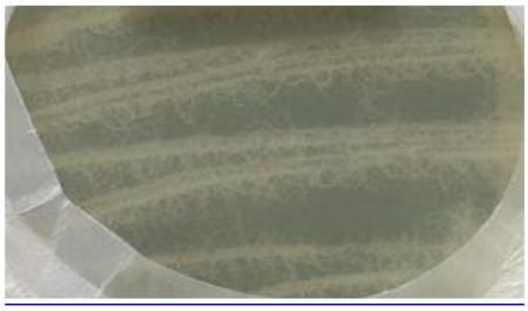

Figure 1. Morphology of *B. mycoides* isolate at 48 hours on NA culture with rhizoidal and milky white colony

Figure 2. DNA products of *B. mycoides* amplified by PCR, M = 1 Kb DNA ladder

Table 1. BLASTN Results of *B. mycoides* Isolate Against NCBI Database

Isolates	Accession Number	Percentage Identity (%)	Countries
Bacillus mycoides strain KUDC1724	KC414703.1	100	South Korea
Bacillus mycoides strain ARD19	KX023234.1	100	India
Bacillus mycoides strain TF3-30	KJ127234.1	100	People's Republic of China

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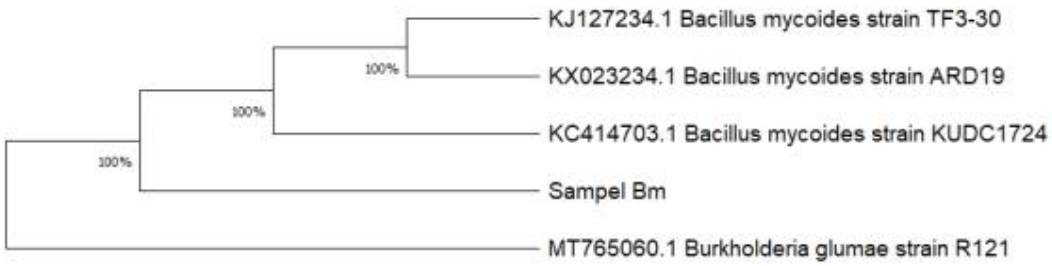

Figure 3. A phylogenetic tree of *B. mycoides* shows the genetic relationship between *B. mycoides* isolate compare with other *B. mycoides* isolates available in GenBank (NCBI)

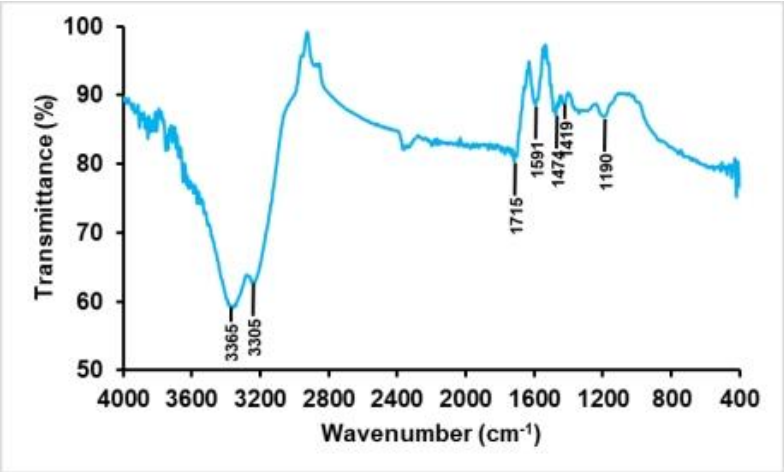

Figure 4. ATR-FTIR transmittance spectrum of *B. mycoides* isolate

Table 2. Assignment of FTIR spectrum of *B. mycoides* isolate

Wave number (cm^{-1})		Molecular vibration of functional groups	Biomolecule contributor	Reference
Experiment	Literature			
3365	3700-3000	O–H and N–H stretching vibrations of hydroxyl groups and amide A	Protein	Bombalska et al. (2011)
3305	~3300	N–H stretching (amide A) of proteins	Protein	Lasch and Naumann (2015)
1715	1715	C=O stretching of esters	RNA/DNA	Lasch and Naumann (2015)
1591	1605-1590	NH2 stretching, C=O, C=N stretching (amide-II band)	Protein	Maity et.al (2013)
1474	1468	C–H deformation of >CH2 (scissoring)	Protein lipid	Davis and Mauer (2010)
1419	1415	C-O-H in plane bending	Carbohydrate, DNA/RNA, Protein	Davis and Mauer (2010)

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Wave number (cm^{-1})		Molecular vibration of functional groups	Biomolecule contributor	Reference
Experiment	Literature			
1190	1200–900	C–OH, C–O–C, C–O ring stretching	Carbohydrate of bacteria cell wall	Grunert et al. (2018), Lasch and Naumann (2015)

Conclusions

This study demonstrates the possibility of spectroscopy FTIR as a method for rapid identification of bacteria isolate. The sample preparation process is simple, the measurement does not take much time and the result reliability makes this method appropriate for routine analysis. The results of this study can be used as initial guesses for the identification of bacterial isolates based on the spectrum parameters obtained.

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