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# Identifications of household's spores using mid infrared spectroscopy

Vivechana Dixit [a], Byoung Kwan Cho [b], Kay Obendorf [a], Jagdish Tewari [a,*]

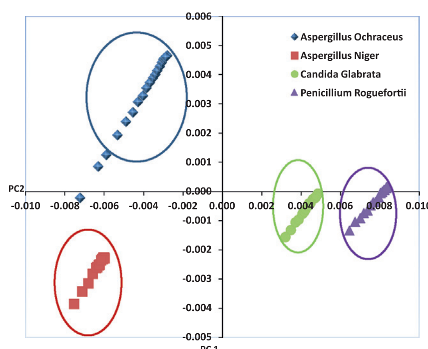
[a] *Fiber Science, Cornell University, Ithaca, NY 14251, USA*

[b] *College of Agriculture and Life Science, Chungnam National University, Yusung-Gu, Daejeon 305-764, Republic of Korea*

## HIGHLIGHTS

- Fungi spectral interpretation.
- Chemometrics modeling of FTIR data for fungi.
- Identification of spores by FTIR conjunction with Chemometrics modeling.
- Application of developed method.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Exposure to household fungi is very common both inside and outside the house and can cause health issues. The application of fourier transforms mid infrared spectroscopy (FTIR) as a screening technique for the detection and identification of household fungi was investigated. Early detection and identification of these household pathogens is very important and critical for their control. The current available methods for identification of fungi are time consuming, expensive and not very specific. Mid IR spectroscopy is a reliable and sensitive technique for the detection of spores. FTIR Spectra of four household fungi such as Aspergillus Ochraceus, Aspergillus Niger, Candida Glabrata and Penicillium Roguefortii were recorded in the mid infrared range from 600 to 4000 $cm^{-1}$ using attenuated total reflectance (ATR) sampling accessory. Chemometrics analysis using principal component analysis (PCA), canonical variate analysis (CVA) and linear discriminant analysis (LDA) were performed to discriminate the fungi samples. Correspondence analysis (CA) was performed in order to visualize the relationship between different spores. An optimum classification of 100% was achieved for four different fungi. Results demonstrated that discriminant analysis of the FTIR spectra of fungi could be used for rapid detection of household fungi.

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## Introduction

Rapid detection and identification of foodborne pathogens is highly important task in food, home safety and in microbiology area. These pathogens are very unique group of spores with over thousand types typically found only in the United States and millions of different types in worldwide.The household fungi produce spores and easily spread in environment and continue to grow in very short period of time. It has been known that fungi doesn't contain chlorophyll therefore unable to make their own food. In order to get food from the host fungi releases specific enzymes to digest fatty acids and proteins.The detection and identification of these fungi are generally carried out using traditional methods and these traditional methods are highly time consuming, expensive, and not specific [1–3]. The infrared region (20–14000 $cm^{-1}$) of the electromagnetic spectrum is divided into three distinct segments: far, near, and mid infrared.The mid-IR or FTIR (400-4000 $cm^{-1}$) is the most commonly used region for analysis as organic molecules possess specific characteristic absorbance

* Corresponding author. Tel.: +1 13174888535.
*E-mail address:* jctewari@yahoo.com (J. Tewari).

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fingerprints in the finger print region of mid IR (800–1800 $cm^{-1}$). Actually FTIR spectroscopy is based on studying the interaction of infrared beam with molecules. As infrared beam interact with a sample, different chemical bonds absorbed infrared light at a specific wavenumbers [4]. Fourier transform infrared spectroscopy is an excellent technique to characterize spores chemical composition.The detection and identification of microorganisms using FTIR spectroscopy has high potential because of sensitivity and can be highly useful for routine screening tool for fungi analysis [5].Mid infrared spectroscopy methods has successfully been applied for identifications of bacteria and yeast [6,7]. This combination with the chemical composition of cells make FTIR tool very powerful tool for detection and identification of fungi [8–14].

## Material and methods

Fungi samples: Aspergillus Ochraceus, Aspergillus Niger, Candida Glabrata and Penicillium Roguefortii strains were obtained from the American Type Culture Collection (ATCC; Manassas, VA) for FTIR measurement.

## FTIR-ATR measurement

FTIR spectra of dried fungi samples were directly collected using Magna Nicolet FTIR spectrometer with a miracle ATR sampling accessory from Pike Technologies (Madison, WI). ATR is based on the phenomenon of total internal reflection, and it gives rise to the biochemical profile of fungi samples. The ATR-MIRacle cell sampling accessory has a single-bounce horizontal ATR (HATR) diamond crystal ~1.5 mm in diameter and a refractive index of 2.34 designed for use in the FTIR spectrometer. The intensity of the ATR spectrum is related to the penetration depth of the evanescent wave into the sample. This depth is dependent upon the refractive index of the crystal and the sample, and upon the wavelength of the IR radiation. Depth of penetration in microns as a function of crystal material used in ATR. Typically the penetration depth is calculated for a sample with a refractive index of 1.5 at 1000 $cm^{-1}$. For the diamond crystal the depth of penetration is Depth of Penetration at 45° is 2 μm.

A major advantage of this accessory is that it requires smaller sample volumes (~0.5 mL) compared to the multiple-bounce HATR accessory; however, the single-bounce accessory is less sensitive because of the decreased signal contact with the sample. Sample preparation procedures for the ATR include direct transfer of fungi samples as thin films; stamping techniques where optical plates were lightly pressed against fungi samples; and direct analysis of fungi samples by ATR-FT-IR. External pressure was applied on ATR measurement to ensure good contact of the samples with the diamond ATR crystal.

The FTIR spectrometer is equipped with a deuterated triglycine sulfate (DTGS) detector, operating at 4 $cm^{-1}$ resolution and 0.32 cm/s mirror velocity. One hundred and fifty-six interferograms were co-added before fourier transformation. The instrument was allowed to purge for 5 min with nitrogen gas (grade1) prior to acquisition of the spectra to minimize the spectral contribution due to atmospheric carbon dioxide and water vapor.

Single-beam spectra of all the samples were obtained and ratioed against the background spectrum of air to present the spectra in absorbance units. The spectrum of the blank ATR-MIRacle cell was used as reference. After every measurement, the ATR crystal was thoroughly washed with distilled water and dried, and its spectrum was examined to ensure that sample residues from the previous acquisition were not retained on the crystal surface. Each experiment was replicated minimum 16 times.

## Chemometric analysis

Chemometrics were used to classify the four different species of fungi using WIN-DAS software (Wiley, Chichester, U.K.) [15]. Baseline corrected and area normalized spectra were used in the chemometric analyses using Principal Component Analysis (PCA), canonical Variate analysis (CVA) conjunction with liner discriminant analysis (LDA).

PCA is a very popular and powerful technique for the identification, classification, and other aspects of data evaluation. PCA decomposes the original matrix into several products of multiplication corresponding to the loadings and scores that indicate the variation of the data as well as the degree of fit.

Canonical Variate analysis (CVA) as a second method also applied to discriminates between groups of observations. Canonical Variate scores have successively maximized between-group variance/within group variance and the CV loadings are obtained as eigenvectors of a matrix [15]. The objective of this procedure is to minimize the within-group variance and maximize the between-group variance. The goodness of fit is indicated by the percentage of correct classification. Discriminant models were based on the calibration data and evaluated separately using the validation data set. The correctly classified samples are expressed as a percentage of the total number of samples in the specific groups. The spectra were normalized by dividing the intensity values corresponding to each wavenumber in the spectrum by its standard deviation before analysis.

Linier discriminant analysis (LDA) was used to develop a discriminative calibration model to classify spectra into groups. The distances between each observation were estimated from group centers. Euclidian, Manhattan city block and Mahalanobis distance were used as the distance metric to measure the distance of each spectrum from each group center. The derived quantities such as group centers and covariance matrices were calculated from the transformed observations, and the assignments to the respective class were then made. Spectra were truncated to the range 800–1800 $cm^{-1}$, baseline corrected and area normalized, and input files in wavenumber versus absorbance format with 250 variates. In each case, the generalization capabilities of the model were validated using k-fold cross validation; the data set was divided into k subsets, and k networks are trained and tested. Each time, one of the k subsets is used as the test set and the other k-1 subsets are pooled to form a training set. The average errors across all k trials were calculated (Table 2).

## Correspondence analysis

Average spectra of Aspergillus Ochraceus, Aspergillus Niger, Candida Glabrata and Penicillium Roguefortii were used in the probability matrix as a contingency data matrix to visualize the relationships between fungi varieties by correspondence analysis using XLSTAT v. 7.5.2 (Kovach Computing Services). Correspondence analysis is a statistical visualization method where the term correspondence denotes a "system of associations" between the elements of two sets. The calculated eigenvalues were ranked in order of percent variance as F1 to $F(n\ 2\ 1)$ for $n$ classes, and cluster plots were generated by plotting values of F1 against F2.

## Results and discussion

### *FTIR spectra of fungi*

The interpretation FTIR spectra of fungi and peak assignments are key steps in the FT-IR analysis of any biological samples. Although most fungi spectra look similar on simple visual

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