Detection of *Fusarium oxysporum* Fungal Isolates Using ATR Spectroscopy

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Abstract. Fungi are considered as serious pathogens for many plants, potentially causing severe economic damage. Early detection and identification of these pathogens is crucial for their timely control. The methods available for identification of fungi are time consuming and not always very specific. In this study, the potential of FTIR-ATR spectroscopy was examined together with advanced mathematical principle component analysis (PCA) and statistical linear discriminant analysis (LDA) to differentiate among 10 isolates of *Fusarium oxysporum*. The results are encouraging and indicate that FTIR-ATR can successfully detect different isolates of *Fusarium oxysporum*. Based on PCA and LDA calculations in the region 850–1775 cm⁻¹ with 16 PC's, the different strains from the same fungal genus could be classified with 75.3% and 69.5% success rates using the “leave one out” method and “20–80% algorithm” respectively.

Keywords: FTIR-ATR, *Fusarium*, fungal detection, PCA, LDA

1. Introduction

Fusarium oxysporum has several specialized forms infecting a variety of plants with various diseases of many symptoms such as vascular wilt, yellows, corm rot, root rot, and damping-off [1, 2]. At the seedling stage, infected plants may wilt and die soon after the appearance of symptoms. On older plants, symptoms are generally more apparent in the period between blossoming and fruit maturation [2].

Early detection of phytopathogens is critical since it enables precise and effective tracing and targeting of treatment or prevention [3]. This could save enormous financial losses [1]. FTIR-ATR spectroscopy has, among other methods, been successfully used to detect and identify fungi samples on the levels of genus, species, and isolates [4–8].

Infrared spectroscopy's unique advantages are simplicity, rapidity, and sensitivity [9]. In addition, much information already exists on the spectral bands obtained from FTIR spectra of living cells [10], adding to the promise of the method as a valuable tool for pathogen detection.

Using multivariate techniques such as PCA, LDA, and ANN for data analysis to extract additional information from mid-infrared spectra achieves good results in identifying fungi strains from the same species [6, 7, 11].

Previous studies [6, 11] showed good results in differentiating between fungi genera, species and strains using FTIR-ATR and multivariate analysis techniques like PCA, canonical variate analysis (CVA) artificial neural network (ANN), but using only one or two isolates from the same species. In another study [7], the ability of FTIR-ATR method was examined in classifying six different strains of *Fusarium oxysporum* applying PCA and LDA techniques and a success rate of 81.4% was achieved.

In the present study one step ahead was taken to try and differentiate a larger number of fungi strains of the same species—*Fusarium oxysporum* using FTIR-ATR with PCA and LDA analyses. This represents a great pattern recognition challenge to classify the samples into many classes with such minute spectral differences between the samples as in the case of isolates (strains) from the same species.

In vivo measurement of fungal samples is of great importance. Recently developed modern infrared fibers are now commercially available and this goal can at present be realized. ATR and transmitting fibers are rather similar, sharing the same principle. Thus, evaluating the potential of FTIR-ATR sampling technique in differentiating fungi strains is very important for future in vivo studies using fiber optic sensors.

2. Materials and Methods

2.1. Fungi

The various strains of *Fusarium* used in this study were obtained from the Department of Plant Pathology at the Gilat Experiment Station, ARO, Israel. These fungal strains were isolated from infected plants by scratching from the infected areas of crops. The samples were grown in potato dextrose medium and identified using classical microbiological techniques [2, 7]. The samples were then separated, purified, and suspended in distilled water for spectroscopic measurements [7].

2.2. Sample Preparation

The samples were placed on the horizontal ZnSe ATR crystal, air dried, and measured by ATR spectroscopy [7].

2.3. FTIR-ATR Measurements

The ATR measurements were performed using an FTIR spectrometer (Bruker Tensor 27) in the ATR mode. 128 coadded scans were collected in each measurement within the wave numbers region 600–4000 cm^{-1} , after the samples were dried. The spectral resolution was set at 4 cm^{-1} . The ATR spectra

were corrected for penetration variation, baseline corrected, and vector normalized using OPUS (6.5) software. The measurements were carried out throughout several weeks.

2.4. Statistical Analysis

2.4.1. PCA

PCA is a standard approach for dimensionality reduction [12, 13], widely used in pattern recognition. PCA is an operator projecting high-dimensional data onto a low-dimension subspace which captures the orthogonal directions with the highest variability, enabling description of the data variability using only few PCs [9, 14].

2.4.2. LDA

Following PCA, the LDA was applied [15, 16]. Training and test sets were selected randomly from the database. Examination of the results was performed using two variants of k -fold cross-validation, applied frequently in pattern recognition. The first was 5-folds, that is, 20–80% with 80% of the data used for training and 20% for testing. Each time, additional 20% were used for testing and all remaining data for training. This procedure was performed 20 times, each time with random data partition into 5 groups. The second variant “leave-one-out” [12, 13], usually applied with small amount of data, was used when $k = N$, the number of data points.

3. Results and Discussion

The main objective in this study was to test and evaluate the potential of ATR spectroscopy in differentiating between ten *Fusarium oxysporum* isolates. More than one hundred known isolates of *Fusarium oxysporum* exist; thus, increasing the number of analyzed isolates is considered an important step toward future commercial use of this technique. All previous studies [6, 11] focus on just few species from the same genus, and only few strains from the same species.

Figure 1 shows the mid-infrared absorption spectra of five out of the ten investigated *Fusarium oxysporum* isolates. The others strains show similar trend and are not shown for the clarity of the figure. The major bands are labeled in the figure. The peak at 1076 cm^{-1} arises mainly from carbohydrate and nucleic acid vibrations [17]. Amide I at 1650 cm^{-1} and amide II at 1553 cm^{-1} are dominant in this region. There is a typical lipid band at 1743 cm^{-1} due to the C=O vibration. Other important bands are the glycogen and chitin C–O and C–C stretching vibrations at 1028 and 1151 cm^{-1} , respectively. In the higher wavenumber region (data not shown), the spectra are dominated by water absorption bands which were excluded as a part of the analysis procedure. The lipids CH_2 absorption peaks at 2849 , 2917 , and 3008 cm^{-1} appear in the higher wavenumber region [18].

Comparing to the clear differences found in the spectra of different genera and species [17], the spectra of different strains are of small differences and are blended and overlapping in many absorption bands. Therefore, differentiating and classifying them is a major challenge. Firstly K -means and cluster analysis, which are unsupervised pattern recognition methods, were tried for differentiating the isolates,

Figure 1: Infrared absorption spectra of *Fusarium oxysporum* isolates in the region 850–1775 cm^{-1} , all ATR spectra passed penetration depth correction, baseline correction, and vector normalization.

but the results were poor. Hence, PCA calculation followed by LDA analysis was performed. LDA analysis is a statistical multivariate supervised method used to efficiently discriminate between the various strains. LDA constructs a linear combination of the variables to discriminate between classes. These calculations were performed on different regions of the spectra, with the 850–1775 cm^{-1} region yielding the best results.

Due to the similarity between isolates of the same species, 16 PCs were used in the differentiation procedure, to achieve good differentiation and simultaneously keep the highest loading (PC16 data not shown) meaningful and noiseless [17]. The “leave one out” algorithm and the 20–80% algorithm enabled differentiating the 10 isolates with success rates of 75.7% and 69.5%, respectively. In the “leave one out,” the results were Foxy1 90%, Foxy2 60%, Foxy3 100%, Foxy4 67.7%, Foxy5 40%, Foxy6 84.6%, Foxy7 78.6%, Foxy8 70%, Foxy9 91.7%, and Foxy10 69.2%.

“Leave one out” is a common cross-validation method, extensively explored in machine learning used to estimate the error in small-sized populations. In all experiments the test sets were statistically independent from the training set, which ensures the validation of results [19].

In summary, mid-infrared vibrational spectroscopy in the ATR mode, in tandem with advanced mathematical and statistical methods, provides a good methodology for classification of fungi on the strain level. This method is fully computerized, objective, and simple to use.

The database and numbers of strains should be enlarged in order to improve the statistics and better simulate the actual agricultural problem where tens of isolates of each species exist.

4. Conclusion

Applying PCA and LDA analyses on FTIR-ATR spectra of fungal samples enabled good classification on the level of isolates. This is an encouraging step forward since the spectral differences are minute. The statistics could nevertheless be improved by enlarging the database.

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References

- [1] G. N. Agrios, *Plant Pathology*, Academic Press, New York, NY, USA, 3rd edition, 1988.
- [2] L. Tsrer (Lahkim), M. Hazanovsky, S. Mordechi-Lebiush, and S. Sivan, "Aggressiveness of *Verticillium dahliae* isolates from different vegetative compatibility groups to potato and tomato," *Plant Pathology*, vol. 50, no. 4, pp. 477–482, 2001.
- [3] G. V. Doern, R. Vautour, M. Gaudet, and B. Levy, "Clinical impact of rapid *in vitro* susceptibility testing and bacterial identification," *Journal of Clinical Microbiology*, vol. 32, no. 7, pp. 1757–1762, 1994.
- [4] S. H. Beattie, C. Holt, D. Hirst, and A. G. Williams, "Discrimination among *Bacillus cereus*, *B. mycoides* and *B. thuringiensis* and some other species of the genus *Bacillus* by fourier transform infrared spectroscopy," *FEMS Microbiology Letters*, vol. 164, no. 1, pp. 201–206, 1998.
- [5] M. J. Gupta, J. M. Irudayaraj, C. Debroy, Z. Schmilovitch, and A. Mizrach, "Differentiation of food pathogens using ftir and artificial neural networks," *Transactions of the American Society of Agricultural Engineers*, vol. 48, no. 5, pp. 1889–1892, 2005.
- [6] A. Naumann, "A novel procedure for strain classification of fungal mycelium by cluster and artificial neural network analysis of Fourier transform infrared (FTIR) spectra," *Analyst*, vol. 134, no. 6, pp. 1215–1223, 2009.
- [7] A. Salman, A. Pomerantz, L. Tsrer et al., "Distinction of *Fusarium oxysporum* fungal isolates (strains) using FTIR-ATR spectroscopy and advanced statistical methods," *Analyst*, vol. 136, no. 5, pp. 988–995, 2011.
- [8] T. Udelhoven, D. Naumann, and J. Schmitt, "Development of a hierarchical classification system with artificial neural networks and FT-IR spectra for the identification of bacteria," *Applied Spectroscopy*, vol. 54, no. 10, pp. 1471–1479, 2000.
- [9] M. Diem, P. Griffith, and J. Chalmers, *Vibrational Spectroscopy for Medical Diagnosis*, John Wiley & Sons, New York, NY, USA, 2008.
- [10] D. Naumann, D. Helm, and H. Labischinski, "Microbiological characterizations by FT-IR spectroscopy," *Nature*, vol. 351, no. 6321, pp. 81–82, 1991.
- [11] R. Linker and L. Tsrer, "Discrimination of soil-borne fungi using Fourier transform infrared attenuated total reflection spectroscopy," *Applied Spectroscopy*, vol. 62, no. 3, pp. 302–305, 2008.
- [12] F. Camastra and A. Vinciarelli, *Machine Learning for Audio, Image and Video Analysis*, Springer, London, UK, 2008.
- [13] R. O. Duda, P. E. Hart, and D. G. Stork, *Pattern Classification*, John Wiley & Sons, New York, NY, USA, 2nd edition, 2001.
- [14] A. Zwielly, J. Gopas, G. Brkic, and S. Mordechai, "Discrimination between drug-resistant and non-resistant human melanoma cell lines by FTIR spectroscopy," *Analyst*, vol. 134, no. 2, pp. 294–300, 2009.

- [15] R. A. Fisher, "The use of multiple measurements in taxonomic problems," *Annals of Eugenics*, vol. 7, pp. 179–188, 1936.
- [16] G. M. James and T. J. Hastie, "Functional linear discriminant analysis for irregularly sampled curves," *Journal of the Royal Statistical Society B*, vol. 63, no. 3, pp. 533–550, 2001.
- [17] A. Salman, L. Tsrer, A. Pomerantz, R. Moreh, S. Mordechai, and M. Huleihel, "FTIR spectroscopy for detection and identification of fungal phytopathogenes," *Spectroscopy*, vol. 24, no. 3-4, pp. 261–267, 2010.
- [18] R. K. Dukor, *Handbook of Vibrational Spectroscopy*, John Wiley & Sons, Chichester, UK, 2001.
- [19] A. Elisseeff, T. Evgeniou, and M. Pontil, "Leave one out error, stability, and generalization of voting combinations of classifiers," *Machine Learning*, vol. 55, no. 1, pp. 71–97, 2004.