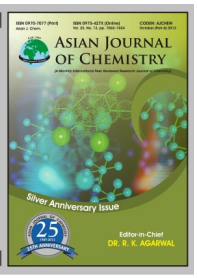
Asian Journal of Chemistry; Vol. 25, No. 15 (2013), 8460-8462

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2013.14790



Characterization of Chinese Cabbage Clubroot by Fourier Transform Infrared Spectra

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(*Received*: 7 November 2012; *Accepted*: 24 August 2013) AJC-13973

Fourier transform infrared (FTIR) spectra have been successfully applied in microbial identification and classification, which provide an alternate method for identification of Chinese cabbage clubroot. Characterization and comparison of the roots and leaves from *Plasmodiophora brassicae* infested Chinese cabbages were performed based on FTIR spectroscopy in this study. Our results showed that the FTIR spectroscopy of leaves is in general similar to that of roots from *P. brassicae* infested Chinese cabbage. However, there was a difference in FTIR spectra between roots and leaves of *P. brassicae* infested Chinese cabbage. In particular, FTIR spectra revealed that 5 peaks at 3380.49, 3011.70, 2854.01, 1744.79, 1244.22 were specific to roots of *P. brassicae* infested Chinese cabbage. This study indicated that FTIR spectra may give a new strategy for rapid identification of Chinese cabbage clubroot.

Key Words: Characterization, Chinese cabbage, Clubroot, FTIR.

INTRODUCTION

The Food and Agriculture Organization of the United Nations has listed Cabbage as one of the top 20 vegetables considered an important food source. However, clubroot caused by the obligate parasite pathogen *Plasmodiophora brassicae* is becoming an emerging threat to the production of cruciferae crops in particular Chinese cabbage and rape in China[1]. The pathogen produces resting spores that can remain infectious for many years, thus, an effective strategy for managing the disease is to avoid planting cruciferous crops in *P. brassicae*-infested soil. Polymerase chain reaction (PCR) protocol has been developed to detect the pathogen in plant and soil samples, which could provide a reliable diagnosis for routine detection of *P. brassicae* in plant and soil materials[2,3]. However, the accuracy of the conventional polymerase chain reaction assay was often affected by false positives and false negatives. Therefore, it is necessary to develop an alternate method for identifying the obligate parasite pathogen.

Recently, fourier transform infrared (FTIR) spectra have been successfully applied in microbial identification and classification[4-7]. FTIR spectroscopy allows the analysis of small quantities of biomass, simultaneous characterization of different functional groups such as lipids, proteins, nucleic acids and polysaccharides in biological molecules and complex structures and without disturbing the systems and requires no consumables or reagents[4,8-10]. However, little information was obtained about the applications of FTIR spectra in obligate parasite pathogen.

The objective of this study was to develop an alternate method to identify the obligate parasite pathogen by examining the FTIR spectra of roots and leaves from *P. brassicae*-infested Chinese cabbages.

EXPERIMENTAL

Roots and leaves of *P. brassicae* infested Chinese cabbage was collected from a vegetable field at Xinmin, Shenyang, China. The identity of the obligate parasite pathogen was determined and confirmed based on the polymerase chain reaction assay as described by Faggian *et al.*[2] and Thomas *et al.*[3].

Sample preparation: Plant samples were stored at -70 ºC until lyophilization. The samples for FTIR analysis were first grounded into fine particles using mortar and pestle. The 1 mg of each sample was then mixed with 100 mg potassium bromide which extensively dried in microfuge tubes using a lyophilizer. These mixtures have been dried for an additional

2 h in the same microfuge tubes. The KBr based pellets were then compressed into a thin disk by establishing pressure of 100 kg/cm^2 (1200 psi) for *ca.* 8 min[4].

FTIR spectroscopy and data analysis: The FTIR spectroscopy data were analyzed as described by Garip *et al*.[4,11] with a small modification. Pellets were scanned at 4 cm^{-1} resolution with 100 scans in the spectral range of 4000-500 cm^{-1} at room temperature. The sample compartment in the FTIR spectrometer was continuously purged with dry air to prevent water vapour. Analysis of the spectral data was performed by using Grams 32 (Galactic Industries, Salem, NH, USA) software. The spectral range of 4000-500 cm^{-1} was analyzed. The band positions were measured according to the center of weight. The averages of the spectra belonging to the same experimental groups, baseline correction, normalisation and the band areas were obtained by using the same software program. The average spectra and normalisation process were applied only for visual representation of the differences, however for the determination of the spectral parameters and calculation of mean values and statistical analysis each baseline corrected original spectrum was taken into consideration.

RESULTS AND DISCUSSION

The FTIR spectroscopy of roots from *P. brassicae* infested Chinese cabbage was summarized in Fig. 1 and Table-1, while FTIR spectroscopy of leaves from *P. brassicae* infested Chinese cabbage was presented in Fig. 2 and Table-2. In general, both the FTIR spectroscopy of roots and leaves show the absorption bands due to O-H (ol/phenol), the O-H (carboxylic acid), the C-H (vinyl group), the N=O (nitrocompound) and S=O (sulfoxide) group, indicating that these peaks may be specific for the Chinese cabbage.

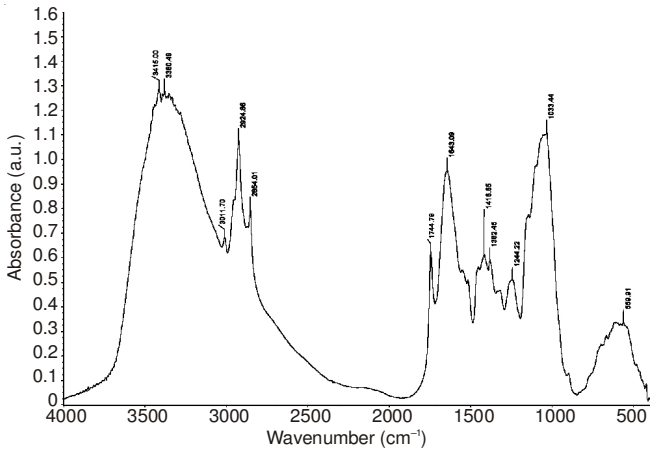

Fig. 1. Average FTIR spectra of *Plasmodiophora brassicae* infested Chinese cabbage roots in the 4000-500 cm^{-1} region

The absorption intensity of roots in these common peaks is in general higher that of leaves from *P. brassicae* infested Chinese cabbage. The absorption intensity of the O-H (ol/phenol) stretching vibration in roots is 1.36 fold than that of leaves; the absorption intensity of the O-H (carboxylic acid) stretching vibration in roots is 2.28 fold than that of leaves, the absorption intensity of the C-H (vinyl group) stretching vibration in roots is 1.11 fold that that of leaves; the absorption intensity of the N=O (nitrocompound) stretching vibration in roots is 1.14 fold that that of leaves; the absorption intensity of S=O (sulfoxide) stretching vibration in roots is 1.57 fold than that of leaves.

TABLE-1
BAND FREQUENCIES AND ABSORPTION INTENSITY OF VARIOUS FUNCTIONAL GROUPS IN *Plasmodiophora brassicae* INFESTED CHINESE CABBAGE ROOTS

Functional groups	Frequency (cm^{-1})	Intensity
O-H (Ol/phenol) stretching vibration	3415.00	1.28
O-H (Ol/phenol) stretching vibration	3380.49	1.26
C-H (Aromatic hydrocarbon) stretching vibration	3011.70	0.67
O-H (Carboxylic acid) stretching vibration	2924.86	1.05
C-H (-CH_2) stretching vibration	2854.01	0.78
C=O (Saturated fat ester) stretching vibration	1744.79	0.50
C-H (Vinyl group) stretching vibration	1416.65	0.60
N=O (Nitrocompound) stretching vibration	1382.45	0.56
C-N (Amine) stretching vibration	1244.22	0.50
S=O (Sulfoxide) stretching vibration	1033.44	1.10

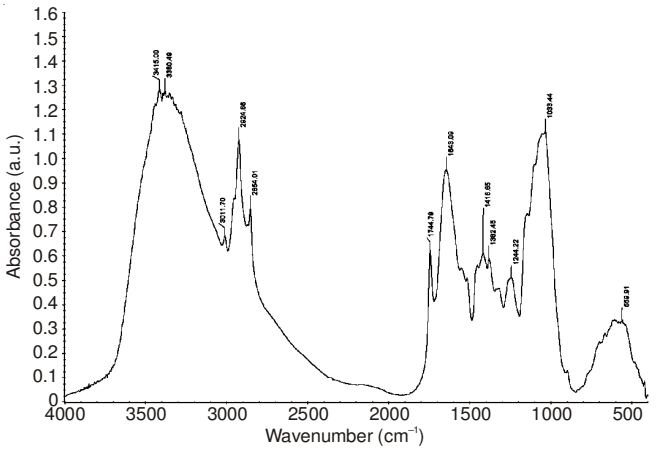

Fig. 2. Average FTIR spectra of *Plasmodiophora brassicae* infested Chinese cabbage leaves in the 4000-500 cm^{-1} region

TABLE-2
BAND FREQUENCIES AND ABSORPTION INTENSITY OF VARIOUS FUNCTIONAL GROUPS IN *Plasmodiophora brassicae* INFESTED CHINESE CABBAGE LEAVES

Functional groups	Frequency (cm^{-1})	Intensity
O-H (Ol/phenol) stretching vibration	3416.74	0.94
O-H (Carboxylic acid) stretching vibration	2927.17	0.46
C=C (Olefin)stretching vibration	1627.05	0.74
C-H (Vinyl group)stretching vibration	1416.99	0.54
N=O (Nitro compound) stretching vibration	1384.63	0.49
N-O (Nitrate ester)stretching vibration	1256.93	0.31
S=O (Sulfoxide)stretching vibration	1068.34	0.70

Results from this study indicated that two distinctive peaks around at 1627.05 and 1256.93 cm^{-1} was only observed in leaves of *P. brassicae* infested Chinese cabbage, but not in roots of *P. brassicae* infested Chinese cabbage. However, five distinctive peaks around at 3308.49 (the C-H (aromatic hydrocarbon) stretching vibration), 3011.70 (C-H (aromatic hydrocarbon) stretching vibration), 2854.01 (the N-H (aminium salt) stretching vibration), 1744.79 (the C=O (saturated fat ester) stretching vibration) and 1244.22 cm^{-1} (C-N (amine) stretching vibration) was only observed in roots of *P. brassicae* infested Chinese cabbage, but not in leaves of *P. brassicae*

infested Chinese cabbage. The five characteristic peaks may be specific to Chinese cabbage clubroot. Therefore, it could be suggested that these characteristic peaks may be able to be used for the identification of Chinese cabbage clubroot.

Previous related reports have revealed that the prominent peak centered at 3308.49 and 3011.70 cm^{-1} is mainly due to aromatic hydrocarbon, the prominent peak centered at 2854.01 cm^{-1} is mainly due to amine derivatives, the prominent peak centered at 1744.79 cm^{-1} is mainly due to saturated fat ester, the prominent peak centered at 1627.05 cm^{-1} is mainly due to olefin, the prominent peak centered at 1256.93 cm^{-1} is mainly due to nitrate ester and the prominent peak centered at 1244.22 cm^{-1} is mainly due to amine derivatives[4-12].

In summary, our results indicated that there was a difference in FTIR spectra between roots and leaves of *P. brassicae* infested Chinese cabbage. Compared to the FTIR spectra of leaves, roots' FTIR spectra have more peaks. In particular, several specific characteristic peaks were determined for Chinese cabbage clubroot by comparing the FTIR spectra between roots and leaves of *P. brassicae* infested Chinese cabbage. Early detection of disease is important for the management of clubroot of crucifers' crops. Compared to the traditional time-consuming method, FTIR spectroscopy is easy to implement and is an emergent physico-chemical technique in detecting clubroot. Therefore, result from this study may give a new strategy for the rapid detect of Chinese cabbage clubroot.

ACKNOWLEDGEMENTS

This work was supported by Zhejiang Provincial Natural Science Foundation of China (R13C140001, Y3090150), National Natural Science Foundation of China (31371904, 31271700), Zhejiang Provincial Project (2010R10091), the Fundamental Research Funds for the Central Universities, the Agricultural Ministry of China (nyhyzx 201003029; 201003066; 201303015) and Key Subject Construction Program of Zhejiang for Modern Agricultural Biotechnology and Crop Disease Control (2010DS700124-KF1101; 2010DS700124-KF1203; 2010DS-700124-KF1309).

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