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Conformational study of protein in some species of *Fusarium* by FT-IR spectroscopy

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Abstract - A novel method of estimating conformation of protein secondary structure within *Fusarium* cells under the influence of growth cultures and bodies was studies by Fourier-Transform Infrared (FT-IR) spectroscopy combined with a deconvolution procedure of amide I band. After infrared data collection and curve-fitting process, the percentages of secondary structure components (a-helix, β-sheet, turn, random coil and β-antiparallel) were obtained. It showed structural diversity among different *Fusarium* species and variations caused by growth cultures and bodies. The method provides a rapidly quantitative technique to study cell structure of fungi.

Key words: *Fusarium*; FT-IR spectroscopy; protein secondary structure; amide I band.

Fusarium is a filamentous fungus widely distributed on plants and in the soil. The diseases caused by *Fusarium* reduce yield and grade and may also contaminate the grain with fungal toxins (Jurgenson *et al*., 2002; Lutz *et al*., 2003). The conformation of a protein is a critical determinant of its functional properties (Ellepola *et al*., 2005). The detailed study of the secondary structure of the proteins will provide knowledge of structure-function relationships to improve phytopathological researches (Logrieco *et al*., 1993; Subirade *et al*., 1998). However, chemical methods have been found it is difficult to investigate conformational characteristics of proteins within microbial cells (Mohaček-Grošev *et al*., 2001). Nevertheless, FT-IR technique allows to obtain direct and non-invasive analysis of protein secondary structure based on the amide I region, composed of C=O stretching vibrations in the region of 1700-1600 cm^{-1} (Oberg *et al*., 2004).

The aim of this paper was to determine protein secondary structure of some *Fusarium* species towards different growth cultures and bodies.

Five fungal isolates were collected from roots of plants at Dongtan, which is the biggest wetland at mouth of the Yangtze River. These isolates were examined after 3 to 10 days of incubation at 26 °C on Potato Dextrose Agar plates (Sigma) in daylight by accepted methods of mycological laboratory practice and by using current literature and determination keys (Nelson *et al*., 1983; Wang *et al*., 1996) and were identified as *Fusarium graminearum*, *Fusarium moniliforme*, *Fusarium nivale*, *Fusarium semitectum*, and *Fusarium oxysporum*. All of the fungal strains were stored in 10% glycerol at -80 °C until tested.

Fusarium strains were cultivated in Czapek Dox Broth (CDB, Sigma) and Potato Glucose Broth (PDB, Sigma) at 26 °C. The mycelia were harvested in the late log growth phase, washed thrice in physiological saline (0.9% NaCl) to remove medium and spores. For sporulation fungal strains were cultured on Oatmeal Agar plates (Sigma). After incubating at 30 °C for two week, the cultures were suspended in 4 to 5 ml of physiological saline by flooding the agar plate, and then the solutions were gently stirred with a glass rod. Spores were harvested through filtration, and also washed thrice. After being centrifuged, all samples were desiccated under vacuum (0.1 bar) for several hours, and a small piece of dried samples was milled with KBr into powder and pressed into a pellet.

FT-IR spectra were recorded on a Thermo Avatar System 370 spectrometer equipped with a DTGS detector. The spectrometer system was controlled by an IBM-compatible PC running OMNIC software (version 5.1). The spectra were recorded between wavenumbers of 4000 to 400 cm^{-1}. 64 interferograms were collected in transmission mode with 4 cm^{-1} resolution and co-added to improve the signal-to-noise ratio of the spectrum.

Analysis of the protein secondary structure of samples was carried out on the basis of the shape of the amide I band, located at 1600-1700 cm^{-1}. Secondary derivative and Fourier self-deconvolution enhancement were applied to estimate the position and width of component bands in the region of 1700-1600 cm^{-1}. Based on these parameters curve-fitting process was carried out by GRAMS/AI software (version 7.02, Thermo Galactic) to get the best Gaussian curves that fit the original spectrum (GRAMS/AI

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TABLE 1 - Comparison of the protein secondary structure elements in the amide I region by curve-fitting

Fusarium strains	Relative contribution (%) of secondary structure elements*				
	β-anti	turn	α-helix	random coil	β-sheet
Mycelia collected from CDB					
F. graminearum	6.4	13.2	42.1	5.7	32.7
F. moniliforme	17.0	8.8	30.0	8.9	35.3
F. nivale	13.5	21.4	34.8	12.0	18.3
F. semitectum	13.2	20.2	28.1	22.2	16.4
F. oxysporum	15.4	2.3	45.6	3.4	33.4
Mycelia collected from PDB					
F. graminearum	12.1	5.9	45.2	2.7	34.1
F. moniliforme	10.8	25.1	23.5	3.7	36.9
F. nivale	13.1	4.7	48.1	3.7	30.4
F. semitectum	9.3	26.7	25.0	3.1	36.0
F. oxysporum	11.0	10.2	35.9	8.2	34.8
Spores					
F. graminearum	14.0	16.5	13.6	37.9	18.0
F. moniliforme	8.5	18.1	42.6	2.2	28.7
F. nivale	9.7	18.3	33.0	25.0	14.0
F. semitectum	11.1	21.3	29.9	13.2	24.5
F. oxysporum	16.5	40.3	26.6	1.8	14.8

* The percentages of these secondary structure components were calculated as the ratio of the corresponding peak areas to the total of the amide I peaks.

Guide, 2001). The component bands of amide I were attributed according to the well-established assignment criterion and the peaks corresponding to a-helix (1656-1650 cm^{-1}), β-sheet (1610-1640 cm^{-1}), turn (1660-1677 cm^{-1}), random coil (1640-1648 cm^{-1}) and β-antiparallel (1680-1692 cm^{-1}) were adjusted (Ahmed *et al*., 1995; Paris *et al*., 2005). The area of all the component bands assigned to a given conformation are then summed up and divided by the total area. All FT-IR experiments were performed in triplicate and reproducible data (with standard deviations < 5%) were obtained.

Differences in amide I band profile originate from those in relative proportion of different types of secondary structure. The large amount of existing experimental data allows an assignment of the individual amide I band components with the different environments of the amide group of the protein backbone, directly related to the secondary structure elements of proteins (Ahmed *et al*., 1995; Paris *et al*., 2005). Table 1 lists the analysis of the amide I domain for each sample. The comparison of these decompositions revealed differences in the secondary structures of overall proteins in the cells. The proteins within *Fusarium* cells were the most structured with amide group of peptide backbone involved β-structure (β-anti, turn and β-sheet), compared to α-structure (α-helix) and random coil. For example, *F. nivale* mycelia collected from CDB contained 53.2% of β-structure, 34.8% of α-structure and 12% of random coil. Moreover, all the β-sheet in the protein secondary structure of *Fusarium* cells, except the spores of *F. oxysporum*, was the main constituent in the β-structure.

Overall the conformation of protein secondary structure of *Fusarium* was greatly influenced by various growth substrates and different between mycelia and spores. The infrared spectroscopy combined with multivariate statistical analysis is a potentially quantitative method to study cell structure of fungi.

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