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Structural Differences between *Fusarium* Strains Investigated by FT-IR Spectroscopy

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Abstract—The structural characteristics of *Fusarium* have received attention from both pure and applied scientists. Because many genes and physiological mechanisms are involved in the development of a particular structure type, this research is complicated. For revealing the structure of macromolecule in these fungal cells, FT-IR spectroscopy combined with multivariate statistical analysis was performed to characterize the structure of protein and polysaccharide of spores and mycelia obtained from different culture medium. The second derivative FT-IR spectra exhibited strain-specific infrared characteristics in the protein secondary structure sensitive amide I region. The region between 750 and 950 cm^{-1} assigned to α- and β-glucans was investigated for studied samples. Principal components analysis (PCA) allowed us to separate mycelia into two clusters according to different growth medium, indicating that spectra of strains may have been greatly affected by cultivation conditions.

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Key words: *Fusarium*, FT-IR spectroscopy, protein, glucan, chitin, structure

Fusarium is a filamentous fungus widely distributed on plants and in soil. Fusarium head blight (FHB) caused by *Fusarium* reduces yield and quality of grains and can result in contamination of plants with fungal toxins [1]. Over the last several years, FHB has been the most important cereal disease of the eastern Canadian prairies, eastern Canada, and the Midwest USA [2]. As well as being common plant pathogens, *Fusarium* may cause various infections in humans, such as keratitis and mycetoma [3, 4]. Knowledge of the chemical composition of these pathogenic fungi is of primary interest both in order to prevent their expansion and to facilitate epidemiological research. However, since an extraction method can change the chemical composition of a delicate biopolymer and this process is greatly complicated, information on the chemical structure of proteins and polysaccharides produced by *Fusarium* is scarce.

The modern infrared spectroscopic method with high signal-to-noise ratio and good repeatability, combined with multivariate statistical analysis, can be used successfully in the analysis and identification of complex biological materials. FT-IR spectroscopy measures vibrations of functional groups and these chemical fingerprints are determined by the total chemical composition of cells [5]. The intensities of IR spectra provide quantitative information while the absorption positions reveal qualitative characteristics about the nature of the chemical bonds, their structure, and their molecular environment [6]. In the recent years, FT-IR spectroscopy has been sufficiently well developed to enable the application of this technology to study cells and molecules that play important roles in the field of microbiology [7-9].

The aim of the present study was to increase knowledge on structural diversity of *Fusarium* mycelia and spores using FT-IR spectroscopic techniques. Here the structural characteristics of some fungal cell components from mycelia collected from different culture media and spores are reported.

MATERIALS AND METHODS

Fungal strains and growth conditions. Five isolates belonging to *F. graminearum*, *F. moniliforme*, *F. nivale*, *F.*

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semitectum, and *F. oxysporum*, respectively, were collected from roots of common plants. The *Fusarium* isolates were identified after 3 to 10 days of pure culture at 26°C on Potato Dextrose Agar plates (Sigma, USA) in daylight. These isolates were examined by colonial aspects and microscopic morphology [10, 11]. All of the isolates were stored in 10% glycerol at −80°C until tested.

The fungal strains were cultivated in Czapek Dox Broth (CDB) (Sigma) and Potato Dextrose Broth (PDB) (Sigma) at 26°C. The mycelia were harvested in the late log growth phase and washed thrice in physiological saline (0.9% NaCl) to remove medium and spores. For sporulation, the 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 sample was milled with KBr into powder and pressed into a pellet.

Recording of spectra. FT-IR spectra were recorded on a Thermo Nicolet (USA) Avatar-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 wave numbers of 4000 to 400 cm^{-1}. Totally 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. All FT-IR spectra were recorded in triplicate on separately growth cultures (Fig. 1).

Analysis of the FT-IR spectra. SPSS (version 13.0, SPSS Inc.) was employed to perform principal components analysis (PCA) so that dimensionality of the multivariate data could be reduced while performing most of the variance [12]. Before the multivariate statistical analysis, the original spectra were first normalized so that the smallest absorbance was set to 0 and the highest absorbance set to +1 for each spectrum; next, the second derivatives of every spectrum were calculated using a Savitzky–Golay algorithm with nine smoothing points to enhance the resolution of superimposed bands and to minimize problems from unavoidable baseline shifts [9, 13].

RESULTS AND DISCUSSION

Principal components analysis. For all the five *Fusarium* species (Fig. 1), it is shown that the spectra of the mycelia collected from CDB differed somewhat from the mycelia collected from PDB. Figure 2 shows the score plot resulting from the principal components analysis based on the entire FT-IR wavelength region. Two main clusters can be clearly observed; one cluster contains only strains grown on CDB while the other

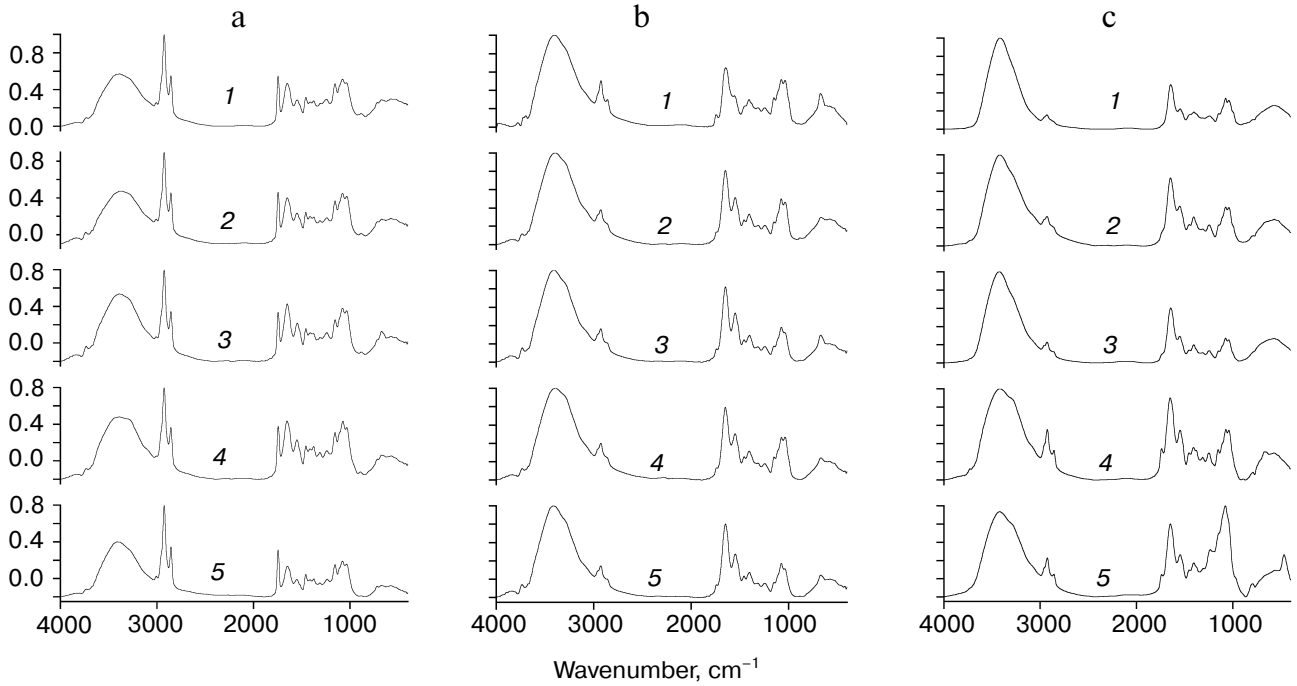

Fig. 1. Comparison of the normalized FT-IR spectra of *Fusarium*. Spectra of mycelial samples collected from CDB (a) and PDB (b) and spectra of spores (c). *1-5*) *F. oxysporum*, *F. semitectum*, *F. nivale*, *F. moniliforme*, and *F. graminearum*, respectively.

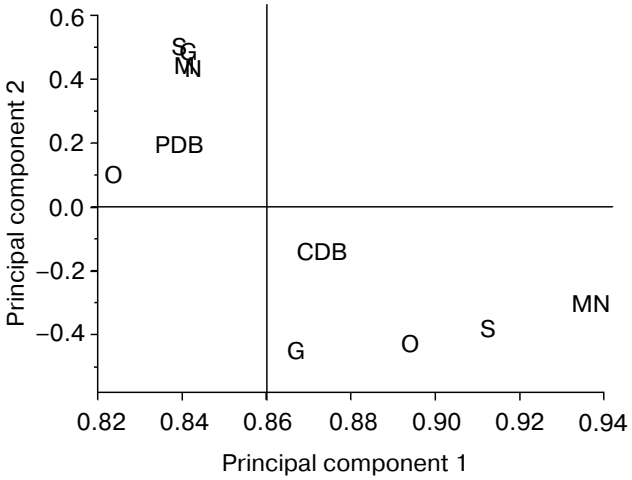

Fig. 2. Plot of component loadings for principal component 1 versus principal component 2 for FT-IR spectra from *Fusarium* mycelia cultivated on two different media, CDB and PDB. The first and second principal components explained 76.0 and 18.2% of the total variance, respectively. G, *F. graminearum*; M, *F. moniliforme*; N, *F. nivale*; S, *F. semitectum*; O, *F. oxysporum*.

cluster comprises all strains grown on PDB. Most probably, these spectral variations are due to changes of the cell wall composition and protein expression [5], since the fungal spectra are dominated by different growth medium. The results obtained by FT-IR are quite consistent with previous spectroscopic studies of microorganisms [14]. The score plot also shows that the variations in the cluster of each mycelium from CDB were larger than the variations within mycelial cluster caused by growth on PDB. To maximize the sensitivity of the FT-IR method for identification of *Fusarium*, it is still necessary to select one growth medium for cultivation of samples [14]. To our knowledge, the differentiation of FT-IR spectra of *Fusarium* towards changes in the growth medium has not been reported before. The result implied that the FT-IR spectra of the mycelia collected from CDB is more advantageous to separate these fungal species for identification, but more time would be required for cultivation.

Overall protein secondary structure. The infrared spectra of polypeptides in cells exhibit a number of so-called amide bands I and II contours between 1500 and 1700 cm^{-1}, which represent different vibrational modes of the peptide bond. Figure 3 depicts typical absorption spectra of *F. graminearum* obtained from fungal mycelia collected from CDB (a) and PDB (b) and spectrum of spores (c).

The amide I band (between 1600 and 1700 cm^{-1}) is mainly associated with the C=O stretching vibration and is directly related to the backbone conformation [15]. In the spectra of *F. graminearum*, at least two amide I absorption maxima could be observed between 1630 and 1660 cm^{-1}, which are sensitive to hydrogen bonding and coupling between transition dipole of adjacent peptide bonds and hence indicates different secondary structure elements [16, 17]. The amide II derives mainly from in-plane N–H bending coupled to C–N and the C–C stretching vibrations [18]. As shown in Fig. 3, the amide II was found between 1500 and 1560 cm^{-1} region and it is more complex than amide I. The absorption maximum of the amide II is located near 1545 cm^{-1}, nevertheless the amide II absorption frequency was shifted from 1543 to 1551 cm^{-1} when *F. graminearum* was cultured in different culture medium (Fig. 3). Regardless of

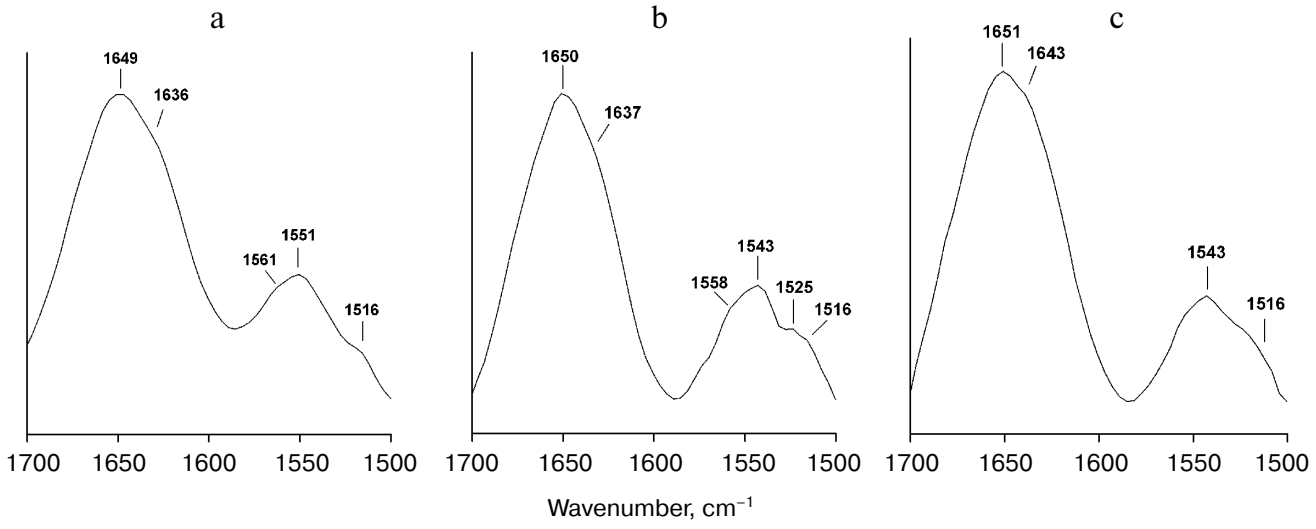

Fig. 3. Typical FT-IR spectra of *F. graminearum* in the amide bands I and II regions. Spectra of mycelial samples collected from CDB (a) and PDB (b) and spectrum of spores (c).

sample sources, all spectra exhibited a constant feature at 1516 cm^{-1}, which is assigned to a tyrosine side chain vibration.

Generally, the widths of amide bands I and II are greater than adjacent peak-to-peak separation, these broad bands in IR spectra are complex composites of overlapping components representing the differently important secondary structure elements. However, the resolution-enhanced spectra allow the identification of the various secondary structures present in proteins and peptides of *Fusarium* strains [19, 20]. The second derivative spectra obtained for different *Fusarium* samples are shown in Fig. 4. Most of the peak positions were easily found in the second derivative spectra. The spectra of *Fusarium* from 1600 to 1700 cm^{-1} (Figs. 4a and 4b) indicated that all mycelial samples are characterized by intense β-antiparallel absorption bands between 1680 and 1691 cm^{-1}, β-turn absorption bands between 1658 and 1678 cm^{-1}, all positions of α-helix absorption bands near 1651 cm^{-1}, all positions of random coil absorption bands around 1643 cm^{-1}, and β-sheets absorption bands between 1622 and 1643 cm^{-1}. In the amide II region (1500 to 1600 cm^{-1}), the mycelial samples shows eleven main absorption features near 1593, 1585, 1774, 1566, 1558, 1551, 1543, 1531, 1521, 1516, and 1507 cm^{-1}. The presence of these bands can be attributed to amino acid side chains and secondary structure elements. These band assignments are given elsewhere [21-23].

Although there are some changes in the spectra of *Fusarium* mycelia in growth medium from CDB to PDB, the effect of variations in the growth medium was slight on the whole (Figs. 4a and 4b). However, spectra of spores demonstrate two additional peaks in the 1693-1697 cm^{-1} and 1500-1504 cm^{-1} wave number regions, which are not present in the spectra of mycelial samples. The occurrence of these additional peaks is accompanied by small differences in the region of 1607-1636 cm^{-1} within the spectra of spore samples. Moreover, peaks

Table 1. Comparison of the main near bands from *Fusarium* samples, glucan, and chitin (1000-4000 cm^{-1})

Glucan	Chitin	*F. graminearum**	*F. graminearum***	*F. graminearum****	*F. moniliforme**	*F. moniliforme***	*F. moniliforme****	*F. nivale**	*F. nivale***
3420 vs, vb	3475 vs, vb	3421 vs, vb	3419 vs, vb	3420 vs, vb	3400 vs, vb	3400 vs, vb	3420 vs, vb	3387 vs, vb	3406 vs, vb
	3265 vs, vb	3274 vs, sh	3266 vs, sh	3278 vs, sh	3272 vs, sh	3267 vs, sh	3252 ws, sh	3318 vs, sh	3300 vs, sh
2955 w, sh	2963 m	2959 m, sh	2960 m, sh	2958 m, sh	2960 m, sh	2954 m, sh	2955 m, sh	2952 m	2957 m
	2933 m	2935 vs			2945 s, sh				
2925 s		2920 vs	2927 m	2925 m		2927 m	2926 s	2924 s	2927 m
	2893 m			2897 m, sh					
2875 m	2877 m	2877 s, sh	2878 w, sh	2874 w, sh	2871 s, sh	2878 m, sh	2874 m, sh		2876 w, sh
2855 m		2854 vs	2856 w	2854 m	2855 vs	2855 w, sh	2854 m	2854 m	2854 w
1731 m, sh		1747 vs, b	1733 w	1745 m, sh	1745 vs, b	1735 w	1732 m	1747 m	1748 w
1713 m, sh		1716 w	1716 w	1716 m	1716 m	1716 w, sh	1714 m, sh	1716 w	1716 w, sh
1642 m	1664 vs	1651 s, b	1651 vs, b	1651 vs, b	1651 vs, b	1651 vs, b	1653 vs, b	1651 vs, b	1651 vs, b
1558 w	1560 vs	1558 m	1558 s	1550 m	1558 s	1558 s	1552 s	1557 s	1558 s
1461 m		1458 m	1458 m	1462 m	1458 s	1458 m	1458 m	1458 w	1458 m
1422 m	1429 m	1416 m	1419 m	1427 m, sh	1419 s	1423 m, sh	1415 m	1419 w	1419 m
	1391 m	1396 m	1396 m	1388 m, sh	1396 m	1392 m, sh	1396 m	1396 w	1396 m
1376 m	1377 s	1377 m	1377 w	1377 m	1377 s	1378 m, sh	1377 m	1377 w	1377 m, sh
1316 m	1314 m,b	1315 m	1315 w	1315 m	1315 m	1316 w	1316 m	1315 w	1315 w
1260 m	1262 m	1259 m, sh	1261 w,sh		1256 m, sh			1256 w, sh	1256 w, sh
1230 m	1232 w, sh	1238 m	1242 w	1236 s	1237 m	1242 w	1250 m	1242 w	1242 w
1204 m	1206 m	1207 m, sh	1209 w	1204 s, sh	1203 m	1206 w, sh		1207 w, sh	1210 w, sh
1160 s	1158 s	1157 s	1153 w	1157 s	1157 vs	1177 m	1155 m	1157 w	1153 w
1113 s, sh	1118 s	1112 s, sh		1100 s	1115 vs	1103 m, sh	1106 m	1111 w, sh	1105 m, sh
1078 vs	1074 s, b	1080 s	1076 m	1077 vs, b	1076 vs, b	1077 m, b	1075 s	1080 m	1076 m, b
1042 vs	1030 s	1037 s, b	1034 m, b	1046 vs, b	1034 vs	1033 m	1042 s, b	1035 m, b	1034 m
1000 s, sh						1000 w, sh	1003 m, sh		

Note: Absorptions are described as follows: very strong (vs), strong (s), medium (m), weak (w), shoulder (sh), very broad (vb), and broad (b).

* Mycelial samples collected from CDB.

** Mycelial samples collected from PDB.

*** Spores.

between 1653 and 1675 cm^{-1} are not obvious in the spectra of spores of *F. oxysporum*, and peaks of spores of *F. nivale* between 1508 and 1560 cm^{-1} significantly differ from those in other spectra of spores of *Fusarium*. The above-mentioned spectral features are illustrated in Fig. 4c and indicate that more conformational variations in the peptide backbone are appeared among spores of *Fusarium*.

Carbohydrates. The cell wall is probably one of the most characteristic structures of fungi and can be used to define this group of organisms [24]. Chemical analysis revealed that the cell wall of *Fusarium* contains 74.5% carbohydrate, 4.5% protein, 3.0% lipid, 4.5% ash, and 0.3% phosphorus [25]. In other words, the major part of the polysaccharides content is located in the cell wall. Structurally, the fungal cell wall appears as a biphasic system consisting of two kinds of structural polysaccharides, chitin and glucan that are organized as microfibril bundles that provide rigidity for the cell, and an amorphous component, primarily glycoproteins and other polysaccharides that cover the inner, microfibrillar layer [26]. The spectra of *Fusarium* samples are shown in Fig. 1 and main band positions compared to previous optical studies on glucan and chitin are listed in Table 1 [27].

Comparing spectra in Fig. 1, one finds great resemblance in the spectra of mycelia collected from CDB, particularly in the regions 2750-7850 and 1800-1700 cm^{-1}. Infrared spectra of these fungal cells contained very strong absorbance bands around 2925 cm^{-1} (CH_2 asymmetrical stretching vibrations and C–H stretching vibrations), 2855 cm^{-1} (CH_2 symmetrical stretching vibrations and C–H stretching vibrations), and 1747 cm^{-1} (carboxyl ester C=O stretching vibrations). These bands mainly result from phospholipids [27-29], which are the basic components of cell membrane and cell organelles [26].

There are four important bands occurring at 1731 cm^{-1} (C=O stretching vibrations of acetylated uronic acid),

Table 1. (Contd.)

*F. nivale***	*F. semitectum**	*F. semitectum***	*F. semitectum****	*F. oxysporum**	*F. oxysporum***	*F. oxysporum****	Assignment
3420 vs, vb	3375 vs, vb	3395 vs, vb	3420 vs, vb	3383 vs, vb	3406 vs, vb	3419 vs, vb	O–H stretching
3323 vs, sh	3271 vs, sh	3317 vs, sh	3309 vs, sh	3252 vs, sh	3250 vs, sh	3300 vs, sh	
2958 w, sh	2953 m, sh	2955 m, sh	2955 m, sh	2953 s, sh	2954 m, sh	2955 w, sh	C–H stretching
2928 w	2926 s	2927 m	2928 m	2921 vs	2825 s	2929 w	
2877 w, sh		2879 m, sh	2879 w, sh			2877 w, sh	CH_2 stretching
2854 w	2854 m	2857 w, sh	2858 w, sh	2854 vs	2854 m	2853 w	
1748 w, sh	1747 m	1732 w	1728 w, sh	1747 vs, b	1743 w	1731 w, sh	C=O stretching
1714 w, sh	1716 w	1716 w, sh	1713 w, sh	1717 m	1712 w, sh	1712 w, sh	
1646 s, b	1651 m, b	1651 s, b	1651 m, b	1651 vs, b	1651 s, b	1647 m, b	Amide I
1554 m	1558 w	1558 s	1555 m	1558 m	1550 m	1542 w	Amide II
1454 w	1458 w	1458 w	1461 m	1458 m	1466 w	1454 w, sh	CH_2 bending
	1419 w	1415 m		1422 m	1423 w, sh	1418 w, sh	
1406 w	1396 w	1396 w	1406 m	1396 m	1408 m	1401 w	Pyranose C–C
1381 w, sh	1377 w	1377 w, sh	1379 m, sh	1377 m	1381 m	1377 w, sh	Bending + O–H bending
1315 w	1315 w	1315 w	1313 w	1315 m	1315 w	1314 w	C–O–H bending + CH_2 deformation
	1257 w, sh			1268 m			
1246 w, b	1240 w	1242 w	1246 w	1242 m	1242 w	1244 w	O=S=O stretching
1209 w, sh	1203 w	1209 w, sh	1208 w, sh	1200 m, sh	1209 w, sh	1211 w, sh	
1153 w	1157 w	1169 m	1149 w	1156 s, b	1153 m	1250 w	Pyranose C–C
1106 w, sh	1111 w, sh	1105 w	1105 w, sh	1111 s, sh	1103 m	1103 w, sh	Stretching + C–O stretching + C–H stretching
1078 w	1080 m	1078 m	1076 m	1079 vs, b	1076 s	1076 m	
1043 w, b	1034 m, b	1034 m, b	1041 m, b	1034 s, b	1034 s, b	1045 m, b	
1000 w, sh			999 w, sh		999 m, sh	999 m, sh	

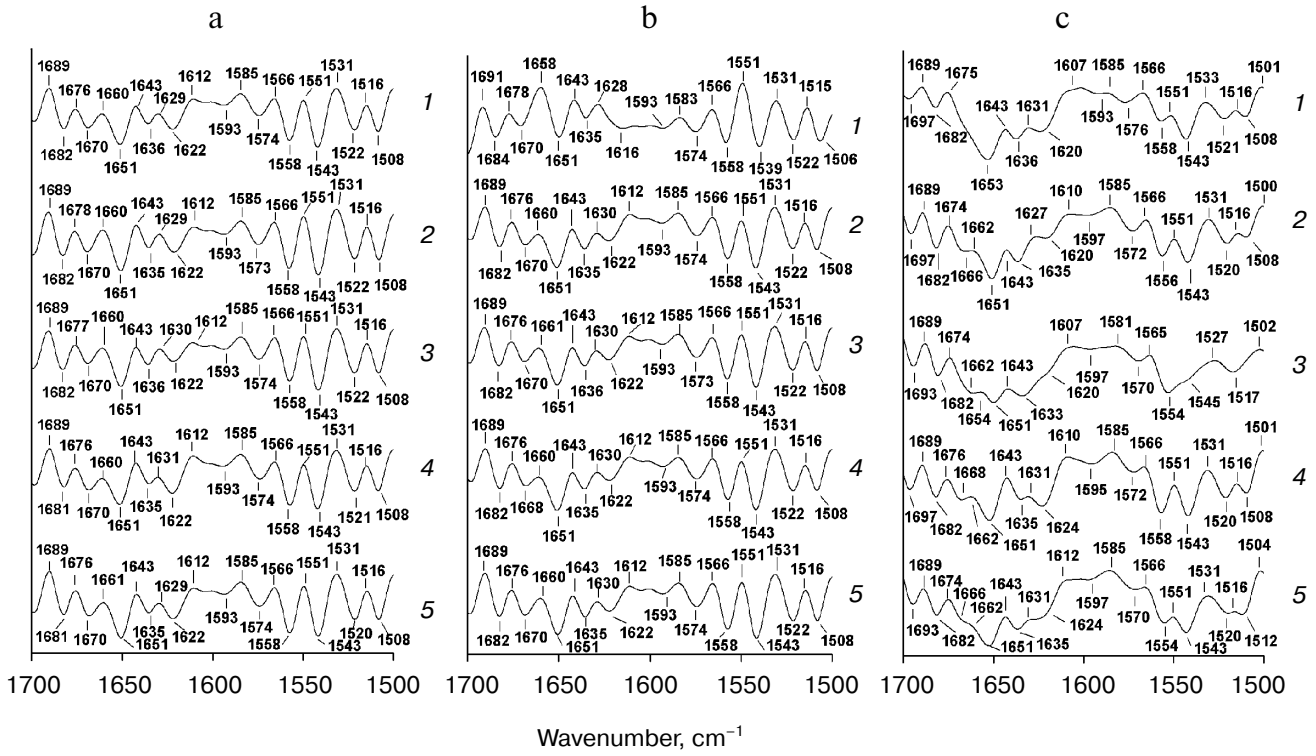

Fig. 4. Comparison of second derivative FT-IR spectra of *Fusarium* in the amide bands I and II regions. Spectra of mycelial samples collected from CDB (a) and PDB (b) and spectra of spores (c). *1-5*) *F. oxysporum*, *F. semitectum*, *F. nivale*, *F. moniliforme*, and *F. graminearum*, respectively.

Table 2. Presence of α- and β-glucan band in the second derivative of FT-IR spectra of various *Fusarium* isolates

Type of glucan	Band, cm^{-1}						
	952	929	926	890	844	822	783
		α-1,6	α-1,3	β-1,3	α-1,3	α-1,3	β-1,3
Chitin	952						
Mycelium grown on CDB							
F. graminearum	956	930	926	889	845	829	779
F. moniliforme	951	930	928	891	842	824	781
F. nivale	953	933	926	889	843	827	781
F. semitectum	952	932	928	889	845	823	781
F. oxysporum	955	933	918	889	841	826	781
Mycelium grown on PDB							
F. graminearum	953	932	926	887	843	821	781
F. moniliforme	956	931	927	891	841	826	779
F. nivale	953	931	927	889	847	823	781
F. semitectum	953	933	926	889	847	824	783
F. oxysporum	956	933	927	891	845	822	779
Spores							
F. graminearum	953	933	925	897	844	822	785
F. moniliforme	953	932	927	892	843	822	784
F. nivale	957	933	926	889	843	823	785
F. semitectum	951	933	926	889	843	823	783
F. oxysporum	953	933	926	887	841	822	783

1713 cm^{-1} (C=O stretching vibrations of protonated uronic acid), 1642 cm^{-1} (amide I), and 1558 cm^{-1} (amide II) in glucan (Table 1). If no fundamental bands were found in that interval, it would be considered that glucan were a polymer completely made of monosaccharide units [30]. Thus, it is very likely that glucan in the fungal cells, as a product of alkali extraction from yeast [31], forms complexes with proteins [27]. This would explain the presence of amide bands I and II in glucan and also would prove glucan to be complexes from the analysis of protein secondary structure above. Furthermore, the bands between 750 and 950 cm^{-1} are used for identification of glucan types [27], and the results are listed in Table 2.

FT-IR spectra and their multivariate statistical analysis of various *Fusarium* forms have their own macromolecular fingerprint. The data can provide some insight into the importance of different chemical information on biopolymer in fungal cells. The determination of macromolecular structure of spore and mycelium provide the bio-infrared spectroscopic researcher with an increased understanding of the role of FT-IR spectroscopy in structural and molecular microbiological investigations. Likewise, this paper will supply structural characteristics of *Fusarium* to facilitate infection control and epidemiological research for biologists. Furthermore, since the chemical composition of the cells is dependent on the cultivation conditions and the standardized growth conditions for identifying *Fusarium* by FT-IR spectroscopy is important, the study presents significant information for identification of these filamentous fungi in general microbiological research.

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