Internet Journal of Food Safety, Vol.13, 2011, p.379-385

Identification of Food Degrading Fungi by Using FT-IR Spectroscopic Analysis of Hydrolytic Enzyme Activities

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Abstract

Fungi show remarkable flexibility in adapting to different culturing environments. They can alter their metabolism to make use of different substrates. Penicillium and Aspergillus are the most economically important genera among filamentous fungi. Much of their economic impact is deleterious, with food spoilage, mycotoxin production and biodeterioration. The objective of this study was to identify and compare hydrolysis properties of various food contaminating fungi (Penicillum ssp. and Aspergillus niger.) based on the whole extracellular hydrolytic product profiles obtained by Fourier Transform Infrared Spectroscopy (FT-IR). The spectra of metabolites produced by microorganisms in a sole carbon or nitrogen substrate of dextrose, pepton, agar, starch (rice, corn, wheat), cellulose, and pectin were registered with a Golden Gate FT-IR Bruker IFS-55 spectrophotometer in the classical range of 4000–400 cm−1. The results were analyzed with GRAMS/AI software and metabolite profiles were tested for mathematical models. Phylo-spectral analysis based on the number and the location of the spectral bands revealed further homologies among fungal cultures tested.

Key words: Infrared spectrometry; FT-IR; broth culture; fungi

Introduction

Mould infestation of agricultural products is common in feed and food supplies worldwide, and this can result in enormous economic losses and health threat with toxin contamination (Basaran, 2010). Development of a reliable, rapid, and reproducible detection with a high discrimination of closely related fungal species is of great interest. The conventional methods for identification and species typing of the filamentous fungi exclusively rely on microscopic observations, and key morphological characteristics of hyphae and spores on various culture medium, which may take nearly 1-2 weeks (Ozcelik, 2010). Furthermore, classification of some species based on phenotypic analysis sometimes needs to be amended, because although species show morphological similarities, they may be genetically divergent isolates (Ehrlich et al., 2003; Wang et al., 2001). The DNA based microbial fingerprinting methods (e.g., amplified fragment length polymorphism (AFLP), randomly amplified polymorphic DNA (RAPD) and pulsed-field gel electrophoresis (PFGE)) are currently restricted as they are sometimes incapable of discriminating closely related species, and polymorphism based genome comparisons demand the initial knowledge of genome sequences (Hill, et. al., 2004; Willse et al., 2005).

Fourier-transform infrared spectroscopy (FT-IR) studies have successfully been carried out to identify and characterize a number of bacteria and yeast (intact or fractured) at strain level (Naumann et al., 1991; Timmins et al., 1998; Schmalreck et al., 2000; Al-Qadiri, 2006; Irudayaraj et al., 2002; Lamprell et al., 2006; Gomez et al., 2001; Guibet et al., 2003; Lefier et al., 2000; Holt et al., 1995); however, studies on discrimination of fungi are very limited (Adilson et al., 1998; Erukhimovitch et al., 2005; Fischer et al., 2006). FT-IR differentiates the microorganisms based on diversity in spectral data of chemical compound mixtures (metabolites) produced by microorganisms (Skoog and Leary, 1992). The main advantage of the FT-IR technique is its simplicity in sampling and registration of spectra. Also the technique does not require sample pre-preparation, which means no use of chemicals, it is rapid, cost effective and accurate with high reproducibility.

The major factors that may influence the spectral characteristics of FT-IR spectra are microbial culture conditions and the choice of growth medium. For this reason, it is important to keep culture conditions constant when developing a FT-IR database of spectra. The assessment of genetic variations and microbial diversity are

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associated with extracellular enzyme production and can therefore be analyzed with the use of FT-IR spectroscopy. The main objective of present work was to identify closely related fungal species based on how hydrolytic enzyme activities participate in fungal exploitation of a sole carbon or nitrogen source as observed with FT-IR spectra.

Material and Methods

Fungal Cultures, Isolation, Identification. Cultures of Penicillum allii (1), Penicillum marneffei (2), Penicillum piceum (3), Penicillum expansum (), Penicillum italicum (5), Aspergillus niger PB18 (6) and Aspergillus niger PB29 (7) were isolated from contaminated fruits and vegetables. Isolates were placed on potato dextrose agar (PDA) (Difco, MI, USA) and incubated at 20 ℃. Identification to the species level was performed according to the macroscopic characteristics (colony growth, colony diameter, color, shape, etc.) on PDA and Czapek-yeast-autolysate agar after incubation at 20°–30℃ for 2-7 days, and microscopic analysis of the reproductive structures as described previously (Ilhan et al., 2006). The stock cultures were maintained on PDA slants at 4℃, in the culture collection of the Food Engineering Department at Suleyman Demirel University.

Evaluation of cultivable diversity of fungi on different carbon and nitrogen substrate. For spore production, 7-day-old PDA culture of fungi was flooded with sterile deionized water (10 ml) and spores were harvested by gently scraping the surface. The suspensions were then filtered with two-three layer cheese cloth to remove debris such as mycelia and agar fragments; and they were enumerated using a standard spread plating method on PDA and the plates were incubated at 20℃ for 48 h. The fungal isolates were inoculated separately at a level of 104-105 spores/ml and the inoculated samples were incubated at 20-22 ℃ for 10 days. Growth was done in minimal medium containing following agar (AG) (0.25%), cellulose (CE) (2.5%), pectin (PC) (2.5%), rice starch (RS) ((2.5%), wheat starch (WH) (2.5%), corn starch (CO) (2.5%), dextrose (PO) (0.5%), bovine serum albumin (BS) (0.5%) and peptone (PE) (2.5%) as sole carbon or nitrogen source. After that cultures were filtered through a cotton filter, centrifuged at 5000 rpm for 15 min so that the extracellular supernatant was separated from the fungal hyphae andspores. The remaining supernatant was lyophilized for 10 h to form homogenous dry powder for FT-IR analysis.

FT-IR spectroscopy and measurement. The freeze dried sample was blended in the ball blender with KBr (spectroscopic grade, Merck, Uvasol) with a sample/KBr ratio of 2.5/100 mg and pressed into a disc for FT-IR registration. Infrared spectra of the samples were registered with a Perkin–Elmer BX spectrometer with a resolution of 4 cm^{-1}, in the classic MIR (middle infrared) range of 4000 to 400 cm^{-1} (32 scans) which was expected to contain unique molecular fingerprint vibrational bands occurring at the wavelengths of bio-molecular functional groups (Basaran, 2009). Freeze-dried biopolymers with no microbial contamination were used as reference material.

Multivariate Analysis of the FT-IR spectra with GRAMS/AI software. When measuring samples of the same material, it is expected that the spectra will be the same or at least very similar to one another. However, no two spectra would exactly be the same. Each spectrum is slightly different due to changing environmental conditions such as humidity, differences in sample handling, spectrometer drift, and also batch to batch variations in the sample material. Considering a set of spectra of different samples differences are higher, and therefore some spectral correction must be done to compare series of spectra. For that reason all analyzed spectra were baseline corrected with multi-point technique and linear function. Also offset correction was done to remove background noises, and for each spectrum characteristic point at 1840 cm^{-1} was set to have intensity value equal to 60. Spectra were divided into five groups (3000-2830 cm^{-1}; 1765-1581cm^{-1}; 1484-1210cm^{-1}; 1190-955cm^{-1}; 950-830cm^{1}) and spectral differences were evaluated within each group. Five spectral ranges were determined mainly due to band vawenumber, intensity and shape. Together five characteristic spectral ranges within 72 spectra were compared and the qualitative and partially a quantitative analysis were done. The ratio of intensities of the same two bands but not absolute intensities of given band occurring in different spectra was considered, to determine qualitative changes, for the reason that amount of sample in the disc was random.

Table 1A shows the critical values that are computed based on mathematical model of the probability structure of the multiple comparisons.

Table 1A. Statistical models values developed for the analysis of nine growth medium

Factor #	Eigen value	REV	F-Ratio (REV)	F- Test (REV)	Avg. Predict	Total % Varian	Malinowski's I	Imbedded Error	Real Error (RE)
1	0.6176 E-5	1.90E-10	22.49	0.99	6.57	61.45	0	0	0
2	0.3130 E-5	1.08E-10	12.83	0.99	11.05	92.60	0	0	0.1 E-4
3+	0.0383 E-5	1.52E-11	1.79	0.82	26.03	96.41	0.53E-6	0.50 E-4	0.6 E-5
4	0.0208 E-5	9.67E-12	1.14	0.71	203.3	98.48	0.40E-6	0.29 E-4	0.5 E-5
5	0.0152 E-5	8.46E-12	1.00	0.68	123.9	100	0	0	0

+ selected model for the evaluation of all fungal species

Table 1B. Mahalanobis distance analysis of *P. expansum* grown in nine selected sole carbon and nitrogen substrates

Sample Name	Score	Spectral Residue	Sample Leverage	Predicted Distance	F-Ratio (Spec.)
AG	0.03	0.17 E-9	0.87	253.52	0.00027
BS	0.47	0.13 E-7	0.41	5.21	1.96942
WH	0.08	0.53 E-10	0.87	332.78	0.00002
CO	0.59	0.65 E-7	0.62	12.63	0.40385
PO	0.47	0.11 E-7	0.55	15.39	1.33597
PC	0.31	0.12 E-7	0.50	15.25	1.82364
PE	0.11	0.11 E-7	0.59	270.93	1.32339

Table 1B summarizes an example model with the results of statistical model variations that have been tested for *P. expansum* FT-IR spectra when grown in nine selected substrates.

Statistical model analysis. The number of conserved and dissimilar bands of seven fungal species grown in seven different substrates was analyzed by using GRAMS/AI 8.00 program (Thermo Scientific, Waltham, MA, USA). Conserved/diverse extracellular components of filamentous fungal species were identified by using mathematical models as described.

For the spectral analysis discriminate calibration type was applied and the cross validation diagnostics were performed for single region. The total number of points evaluated was 3601 with a maximum factor of 5 in sequential file order. Preprocessing of data included mean centering, autobaseline, unit area normalization of spectrum. Mahalanobis Distance analysis was used to produce cluster analysis based on spectral analysis on various carbon and nitrogen sources. The program is an integral part of data processing software (GRAMS/AI 8.00).

Results and Discussion

Penicillum ssp. and *Aspergillus* ssp. are economically important genera among filamentous fungi that cause great damage with toxin production, spoilage and deterioration of the plant food materials (Moss, 2008). The FT-IR spectral features of extracellular biochemical constituents (proteins and polysaccharides, oligosaccharides, etc.) were screened whether various nutritional sources can create a 'fingerprint' for each fungal species.

Spectral of homology and divergence of the FT-IR fingerprint regions were analyzed to detect differences of chemicals produced and exported by seven fungal species into the same medium, which implied that spectra were analyzed according to spectral changes occurring in the sample of seven different culturing medium. For this reason, five spectral ranges (3000-2830cm^{-1}; 1765-1581cm^{-1}; 1484-1210cm^{-1}; 1190-955cm^{-1}; 950-830cm^{-1}) were selected mainly due to band vawenumber, intensity and shape, and spectral differences were evaluated within each group.

In the first range (3000-2830cm^{-1}) various C-H vibrations occur. In second region (1765-1581cm^{-1}) bands generated by C=O group vibrations are present such as C=O group (carboxylic acids, esters, ketons). Third, fourth and fifth regions contain bands generated by stretching, bandings, scissoring, twisting, rocking, wagging vibrations of different groups (e.g. C-H, C-C, C=C, C-OH, and others). In earlier reports, the spectral differences below 1500cm^{-1} was referred as the "fingerprint region" of an FT-IR spectrum (Rodriguez-Saona et al., 2001). The third and fourth regions of FT-IR spectra respectively 955-1190cm^{-1} (polysaccharides) and 1210-1484cm^{-1} (proteins) (Oulahal et al., 2008) were of great interest since polysaccharides and proteins were the sole components of the growth medium in this study. Although samples were dried, very intense and a wide band generated by O-H vibration from water molecules occurred in every spectrum, with the maximum intensity at around 3430cm$^{-1.}$

The group of Agar (AG) medium. Figure 1 presents the spectral analysis of AG (1-7) as an overlap. The range of 3000-2830cm^{-1}, mostly this first region discriminate spectra of agar medium, and according to the number of bands and their wavenumbers three groups of fungi can be created. This region corresponds to C-H of polysaccharides, divided spectra into three groups due to number of occurring bands: SPEC AG (2)(4) containing two bands, SPEC AG (3)(5) containing three bands, and SPEC AG (1)(6) also containing three bands. First intense band occurred at 2925 cm^{-1} in all seven studied spectra. The second band of medium intensity, occurred at 2854cm^{-1} and 2869 cm^{-1}, in SPEC AG (2) and SPEC AG (4), respectively. In spectra SPEC AG (3) and SPEC AG (5) second band occurs at 2900 cm^{-1} while third one of small intensity at 2869cm$^{-1.}$

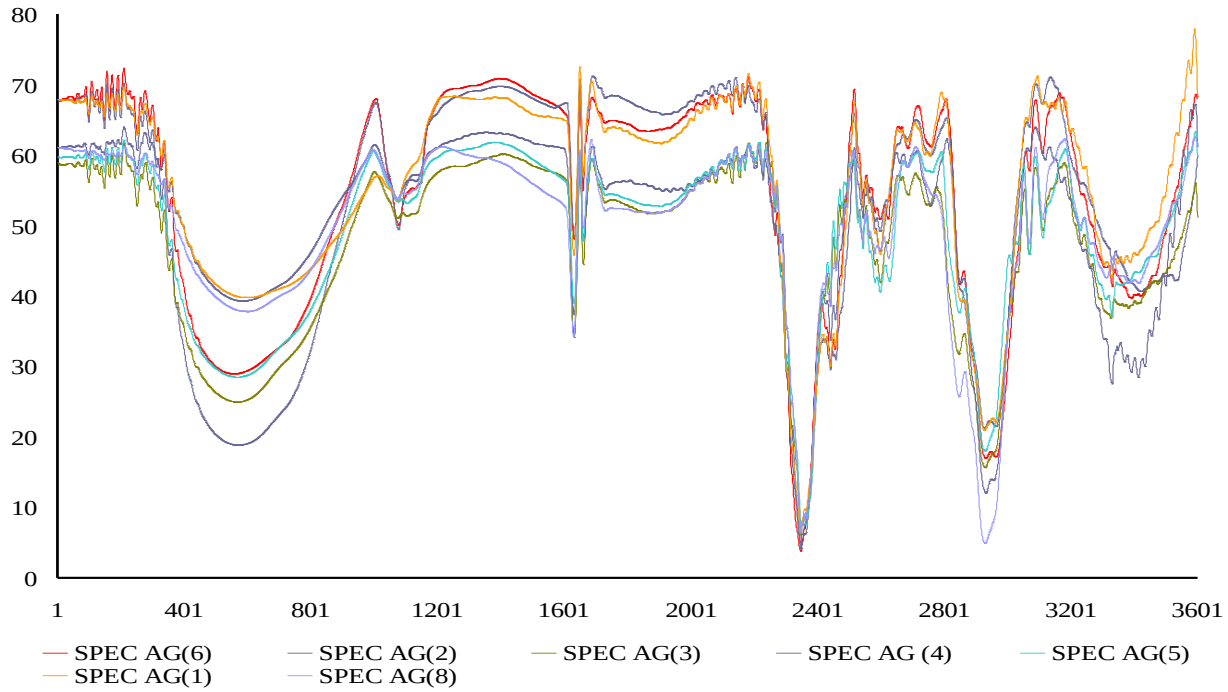

Figure 1. Spectra of agar (AG) (1,2,3,4,5,6,7,8) in whole registered whole spectral range

In the second range of 1765-1581cm^{-1} and the third range of there were two bands located at 1653cm^{-1} and 1638cm^{-1}. Since those bands occurred in all studied spectra, this region was not usable for fungal discrimination. Spectra SPEC BS (1) and SPEC BS (6) to distinguish from six remaining members and each other but there was not sufficient spectral data allowing to distinguish spectra SPEC BS (2)(3)(4)(5)(7) from each other (Figure 2). The bands at 1657 cm-^{1} was attributed to the Amides I vibrations of structural proteins, respectively (Sun et al., 2004).

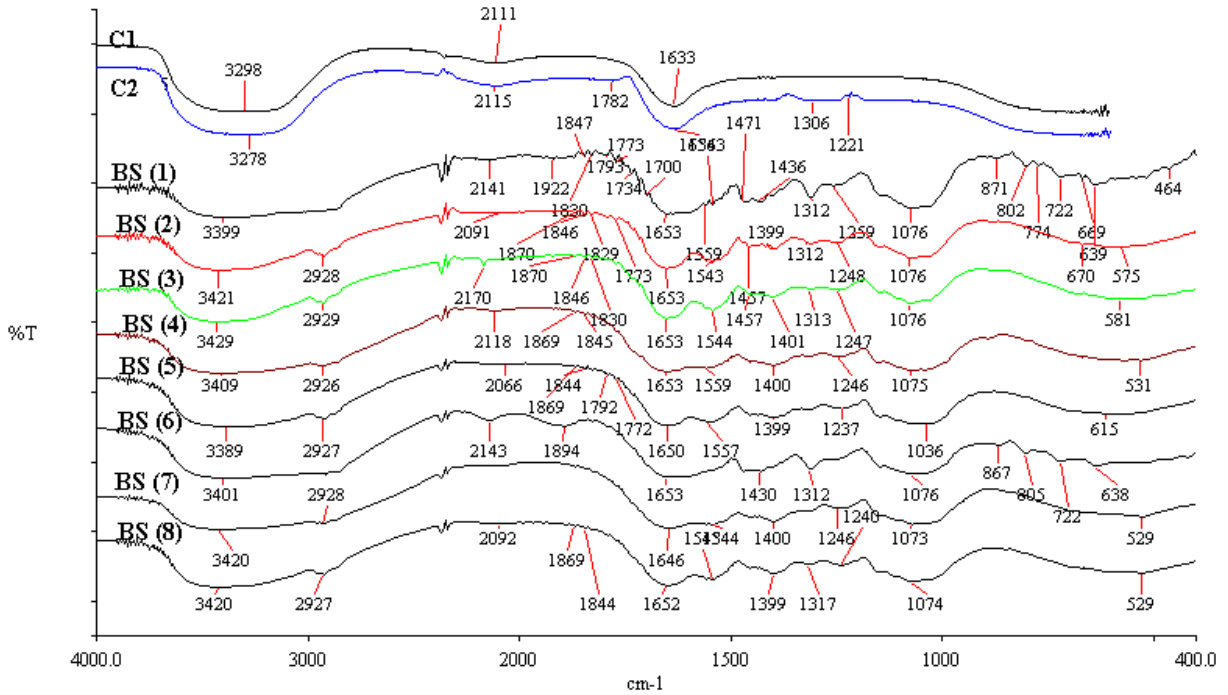

Figure 2. Spectral analysis of bovine serum albumin (BS) (1,2,3,4,5,6,7)

The range of 1190–830cm^{-1} was analogous for all seven spectra and therefore not much of use for analysis of this group of compounds (Figure 3). The data from region 3000-2830cm^{-1}, whereas, generally corresponds to C-H of fatty acids and polysaccharides (Fischer et al., 2006), allowed to distinguish SPEC BS (1) from SPEC BS (6) as in SPEC BS (1) no band at 2956cm^{-1} was detected.

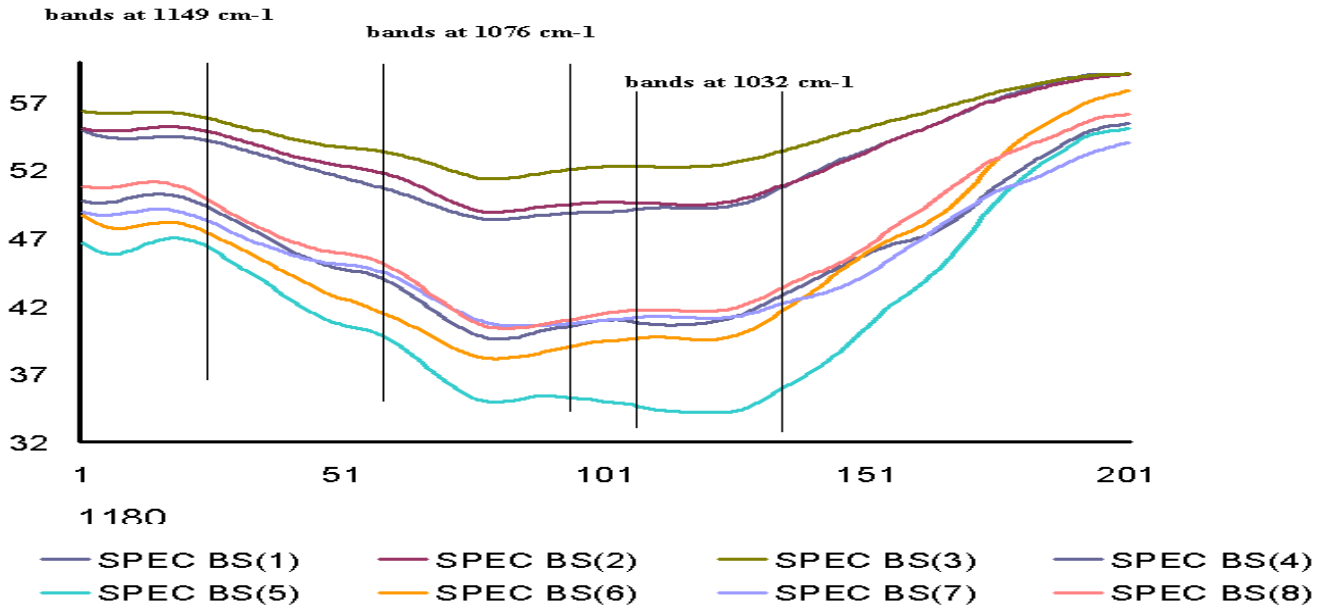

Figure 3. The analogous spectral range of 1190–955cm^{-1} for all eight fungal species in bovine serum albumin (BS)

The group of wheat (WH) rice (RS) and corn (CO) starch medium. The observations for all three starches (wheat, rice and corn) further confirmed that the spectral region of 1200–800cm^{-1} indicates changes in polymer conformation and hydration of starches (Htoon et al., 2009). The band at 1459cm^{-1} was characteristic for all elements from wheat group (WH). In all rice starch (RS group) spectra a band at 2852cm^{-1} was detected.

Spectral data from the high region of 3000-2800cm^{-1} allowed to distinguish SPEC WH (3) from SPEC WH (4) and from other SPEC WH (1)(2)(5)(6)(7) spectra.

In those six spectra two characteristic spectral bands generated by C-H vibrations at 2924cm^{-1} and 2854cm^{-1}. The data in this region 3000-2830cm^{-1} properly distinguish SPEC RS (1) from SPEC RS (7). In case of corn group samples the high region of 3000-2830cm^{-1} and the next spectral region of 1190–955cm^{-1} was not sensitive for changes occurring in metabolite profile did not provide data to distinguish studied samples.

The second region of 1765-1581cm^{-1} contained three characteristic bands at 1745cm 1652cm^{-1}, and 1632cm^{-1} which are present in all studied spectra, therefore this region was not sensitive for any differences occurring in measured samples. The next range of 1765-1581cm^{-1} provided data for distinguishing SPEC CO (6) from other samples.

In the region 950-830cm^{-1} in spectra SPEC WH (3)(4)(6)(7) two common bands emerged at 927cm^{-1} and 863 cm^{-1}, the peak specifically at 930cm^{-1} was due to -C-O-C- in glucose units (Fang et al., 2004). In SPEC WH (6) and SPEC WH (7) only one additional band appeared at 941cm^{-1} which is associated with the

skeletal modes in amylose and amylopectin structures of starch. In the SPEC CO (5) there was not any band, while in SPEC CO(6) there were two very intense bands at 943cm^{-1} and 862cm^{-1}, of the first one indicate the amylose and amiylopectin (Schultz and Baranska, 2007).

The group of pectin (PC) growth medium. In the high region of 3000–2830cm^{-1} the samples of pectin (PC) group generated two bands at 2923cm^{-1} and 2852cm^{-1}, while in SPEC PC (4) and SPEC PC (6) additional single band at 2873cm^{-1} was present. This allowed distinguish those two spectra from others.

In the region 1765-1581cm^{-1} the sample SPEC PC (5) was easy to recognize for its two bands located at 1745cm^{-1} and 1660cm^{-1}. In fact, the major peak observed around 1740-1745cm$^{-1,}$ was generally attributed to culturing medium constitute and the presence of the carboxylic ester in pectin (Wang et al., 2006; Schulz and Baranska, 2007). The latter band 1660cm^{-1} was very characteristic for it was present exclusively in this spectrum.

The next region 1484–1210cm^{-1} was band rich and allowed to distinguish all spectra. The bands located at 1300cm^{-1}, 1277cm^{-1} and 1204cm^{-1} and data in the region of 950-830cm^{-1} distinguished SPEC PE (3) from any other spectrum. The of two bands located 893cm^{-1} and 847cm^{-1},

was the indication of maltose or sucrose disaccharides in the culture (Schultz and Baranska, 2007). The bands detected at 847-852cm^{-1} represents (C–O–C) skeletal mode of α-anomers in the culture medium of pectin (Schulz and Baranska, 2007).

The group of Cellulose (CE) growth medium. Neither of the regions of 3000-2830cm^{-1}, 1765-1581cm^{-1} and 1190-955cm^{-1} was able to distinguish spectra or set it in separate subgroup as similar number of bands in each spectrum occurred. The bands around 1160 cm^{-1} and 1059 cm^{-1} were assigned to the C–O–C asymmetric stretching and cellulose (and hemicellulose) respectively (Kacurakova et al., 2002; Oulahal et al., 2008). In the region 1765-1581cm^{-1}, there was a single strong band at 1655cm-1 in all spectra except SPEC PE (2), and reliable indication of amide I in α-helix secondary structures of proteins (Schultz and Baranska, 2007). In the latter, strong wide band occurred at 1681cm^{-1}. In fact, the region 1780-1485 cm^{-1} spectral zone was attributed to protein (amide I and amide II groups) absorbing region (Schulz and Baranska, 2007). In the region 950-830cm^{-1}, only SPEC CE (1) was distinguished from other species for its band at 903cm^{-1}, which originated from the β-glycosidic linkages between the sugar units in the cellulose and hemicellulose (Sun et al., 2004).

The group of dextrose broth (PO) growth medium. There was one intense and wide band at 1044cm^{-1}, which were expected as bands at 1150, 1110, and 1040cm^{-1} were indicative of the presence of glucose (Gutierrez et al., 1996; Zhao et al., 2005). In the region of 950-830cm^{-1} of each spectrum, the band at 852cm^{-1} is attributed to the vibration of (C–O–C) skeletal mode groups which arise from dextrose in the culturing broth (Schulz and Baranska, 2007). The band at 862 cm^{-1} was reported to correspond the C–C stretching and C–H bending vibrations of the glucosidic rings in starch (Rivero et al., 2009). The common band in all spectra located at 2850-2851cm^{-1} could be an indication of lipids or fatty acids, although this result was not anticipated. In SPEC PO (7) there were four bands, although located at 1680cm^{-1} 1550cm^{-1}, 1660cm^{-1}, and 1550 cm^{-1} could easily select SPEC PO (7).

The group of pectin (PC) growth medium. With peptone containing substrate; there was no or little information to differenciate fungal species. The region of 1484-1210cm^{-1} was rich for the number of bands but was not usable to distinguish samples. Two common bands were located at 1449cm^{-1} and 1401cm^{-1} for all spectra, and this region and assigned to υ(C=O)O- in aspartic acid (Schultz and Baranska, 2007), and probably released due to the hydrolysis of pectin. Specifically the band at 1077cm^{-1}, which was present in all samples, is characteristic of heavy microbial growth, since microorganisms are the source of glycogen or nucleic acids (Oulahal et al., 2008).

Phylo-spectral analysis of fungi. Figure 4 demonstrates the cluster analysis of the seven distinct profiles of Penicillum ssp. and Aspergillus ssp. strains generated from Table 2 modeling analysis. Only two strains of A. niger were closely related and were built an own cluster. P. expansum was affliated with P. italicum than other species. In contrary, P. piceum was distantly related to other Penicillum species by cluster analysis.

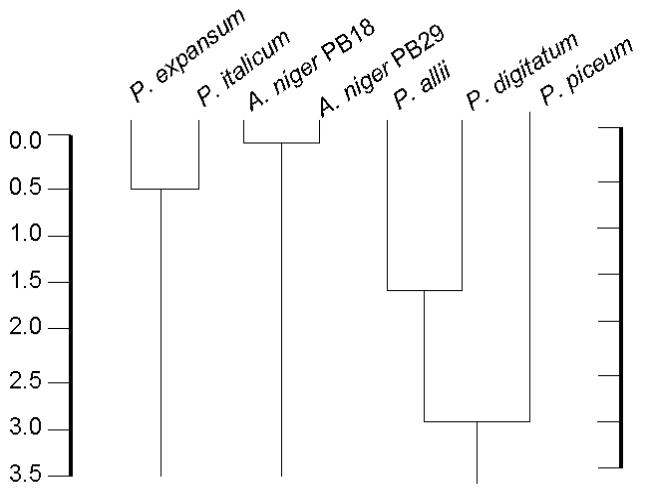

Figure 4. Mahalanobis cluster analysis of hydrolytic enzyme activity of fungal samples grown in eight different carbon and nitrogen sources

FT-IR has potential to be absolute analytical method for microorganism diversification. Fungi are able to utilize a wide variety of carbon and nitrogen sources, less complex substrate would help to standardize cell culturing and make more effective FT-IR differentiation and furthermore produce valuable data for inter-laboratorial comparative studies. FT-IR method for mixture analysis in the literature is generally proposed as a method which requires a reference analytical method. Here it was shown that with proper mathematical modeling, it was possible as absolute analysis to compare and determine fungal species without any reference analysis, and may provide analytical information on biodegradation products of the biopolymers.

References

Adilson R, Esposito E, Benar P. 1998. Evaluation of Panus tigrinus in the delignification of sugarcane bagasse by FT-IR-PCA and pulp properties. J. Biotechnol. 66: 177-185.

Al-Qadiri HM, Lin M, Cavinato AG, Rasco BA. 2006. Fourier transform infrared spectroscopy, detection and identification of Escherichia coli O157:H7 and Alicyclobacillus strains in apple juice. Int. J. Food Microbiol. 111: 73-80.

Basaran P. 2011. Antifungal effect of acids and surface active compounds for post-harvest control of

Aspergillus parasiticus growth on hazelnut. Food Process. Preserv. 35: 236–246.

Basaran P. 2010. Inhibition effect of belzalkonium chloride treatment on growth of common food contaminating fungal species. Int. J. Food Sci. Tech. 48: 515–519.

Ehrlich KC, Montalbano BG, Cotty PJ. 2003. Sequence comparison of aflR from different Aspergillus species provides evidence for variability in regulation of aflatoxin production. Fungal Gen. Biol. 38: 63-74.

Erukhimovitch V, Pavlov V, Talyshinsky M, Souprun Y, Huleihel M. 2005. FT-IR microscopy as a method for identification of bacterial and fungal infections. J. Pharm. Biomed. Anal. 37: 1105-1108.

Fang, JM, Fowler PA, Sayers C, Williams PA. 2004. The chemical modification of a range of starches under aqueous reaction condition. Carbohyd. Poly. 55: 283-289.

Fischer G, Braun S, Thissen R, Dott W. 2006.

FT-IR spectroscopy as a tool for rapid identification and intra-species characterization of airborne filamentous fungi. J. Microbiol. Meth. 64: 63-77.

Gomez M, Bratos Perez MA, Martin Gil FJ, Duenas Diez A, Rodriguez JF, Rodriguez P, Orduna Domingo A, Rodiguez Torres A. 2003. Identification of species of Brucella using Fourier transform infrared spectroscopy. J. Microbiol. Methods 55: 121-131.

Guibet F, Amiel C, Cadot P, Cordevant C, Desmonts MH, Lange M, Marecat A, Travert J, Denis C, Mariey L. 2003. Discrimination and classification of Enterococci by Fourier transform infrared (FT-IR) spectroscopy. Vibrational Spectroscopy 33: 133-142.

Gutiérrez A, Bocchini P, Galletti G, Martinez AT. 1996. Analysis of Lignin-Polysaccharide Complexes Formed during Grass Lignin Degradation by Cultures of Pleurotus Species. Appl. Environ. Microbiol. 62: 1928-1934.

Hill KK, Ticknor LO, Okinaka RT, Asay M, Blair H, Bliss KA, Laker M, Pardington PE, Richardson AP, Tonks M, Beecher DJ, Kemp JD, Kolsto AB, Wong ACL, Keim P, Jackson PJ. 2004. Fluorescent amplified fragment length polymorphism analysis of Bacillus anthracis, Bacillus cereus, and Bacillus thuringiensis isolates. Appl. Environ. Microbiol. 70: 1068-1080.

Holt C, Hirst D, Sutherland A, MacDonald F. 1995. Discrimination of species in the genus Listeria by Fourier transform infrared spectroscopy and canonical variate analysis. Appl. Environ. Microbiol. 61: 377-378.

Htoon A, Shrestha AK, Flanagan BM, Lopez-Rubio A, Bird AR, Gilbert, EP, Gidley MJ. 2009. Effects of processing high amylose maize starches under controlled conditions on structural organisation and amylase digestibility. Carbohyd. Poly. 75: 236–245.

Ilhan S, Demirel R, Asan A, Baycu C, Kinaci E. 2006. Colonial and morphological characteristics of some microfungal species isolated from agricultural soils in Eskisehir province (Turkey). Turk. J. Bot. 30: 95-104.

Irudayaraj J, Yang H, Sakhamuri S. 2002. Differentiation and detection of microorganisms using Fourier transform infrared photoacoustic spectroscopy. J. Mol. Struct. 606: 181-188.

Kacurakova M, Smith AC, Gidley MJ, Wilson RH. 2002. Molecular interactions in bacterial cellulose composites studied by 1D FT-IR and dynamic 2D FT-IR spectroscopy. Carbohydr. Res. 337: 1145-1150.

Lamprell H, Mazerolles G, Kodjo A, Chamba JF, Noel Y, Beuvier E. 2006. Discrimination of Staphylococcus aureus strains from different species of Staphylococcus using Fourier transform infrared (FT-IR) spectroscopy Int. J. Food Microbiol. 108: 125 – 129.

Lefier D, Lamprell H, Mazerolles G. 2000. Evolution of Lactococcus strains during ripening in Brie cheese using Fourier transform infrared spectroscopy. Lait 80: 247-254.

Moss MO. 2008. Fungi, quality and safety issues in fresh fruits and vegetables. J. Appl. Microbiol. 104: 1239-1243.

Naumann D, Helm D,Labischinski H. 1991. Microbiological characterizations by FT-IR spectroscopy. Nature, 351: 81-82.

Oulahal N, Adt I, Mariani C, Carnet-Pantiez A, Notz E, Degraeve P. 2009. Examination of wooden shelves used in the ripening of a raw milk smear cheese by FT-IR spectroscopy. Food Control. 20: 658-663.

Ozcelik S. 2010. Gıda Mikrobiology Laboratuvar Kılavuzu. Isparta. pp.20-40.

Rivero IE, Balsamo V, Müler AJ. 2009. Microwave-assisted modification of starch for compatibilizing LLDPE/starch blends. Carbohyd Polym. 75: 343–350.

Rodriguez-Saona LE, Khambaty FM, Fry FA, Calvy EM. 2001. Rapid detection and identification of bacterial strains by Fourier transform near-infrared spectroscopy. J. Agric. Food Chem. 49: 574-579.

Schmalreck AF, Hotzel H. 2000. Fourier transform infrared spectroscopy, molecular biologic methods and antimyocotic susceptibility patterns for identification and differentiation of Cryptococcus species. Mycoses. 43: 61–68.

Schulz H, Baranska M. 2007. Identification and quantification of valuable plant substances by IR and Raman spectroscopy. Vibrational Spect. 43: 13-25

Skoog DA, Leary JJ. 1992. Infrared absorption spectroscopy. In: Principles of Instrumental Analysis, 4th Ed. (D.A. Skoog and J.J. Leary, eds.) Saunders College Pub, Fort Worth, TX, USA, pp. 252-309.

Sun JX, Sun XF, Zhao H, Sun RC. 2004. Isolation and characterization of cellulose from sugarcane bagasse. Polymer Deg. Stab. 84: 331-339.

Timmins EM, Howell SA, Alsberg BK, Noble WC, Goodacre R. 1998. Rapid differentiation of closely related Candida species and strains by pyrolysis-mass spectrometry and Fourier transform-infrared spectroscopy. J. Clin. Microbiol. 36: 367-374.

Wang LK, Takahasi YH, Kase N, Hanya Y, Yashiro K, Miyaji M, Nishimura K. 2001. Identification of species in Aspergillus section Flavi based on sequencing of the mitochondrial cytochrome b gene. International Journal of Food Microbiology, 71: 75-86

Wang Q, Fan XR, Gao WD. 2006. Characterization of bioscoured cotton fabrics using FT-IR ATR spectroscopy and microscopy techniques. Carbohydr. Res. 341, 2170–2175.

Willse A, Chandler DP, White A, Protic M, Daly DS, Wunschel S. 2005. Comparing bacterial DNA microarray fingerprints. Stat. Appl. Gen. Mol. Biol. 4: 1-19.

Zhao G, Kan J, Li Z, Chen Z. 2005. Characterization and immunostimulatory activity of an (1→6)-a-d-glucan from the root of Ipomoea batatas. Int. J. Immunopharmacol. 5: 1436–1445.