Research Journal of Agriculture and Biological Sciences, 6(1): 1-7, 2010

Biochemical Markers in Taxonomy of the Genus *Fusarium*

Zain, M.E.

Medical Laboratory Sciences Department, College of Applied Medical Sciences,
Al-Kharj University, KSA.

Abstract: The amino acid, fatty acid and secondary metabolite profiles of 11 *Fusarium* were determined. Numerous amino acids including phosphoserine, taurine, glycine, tyrosine, phenylalanine, carnosine, and arginine were detected in all the investigated *Fusarium* species. While aspartic acid, citrulline, valine, cystine, methionine, leucine, histidine and lysine were detected in some *Fusarium* species. Only leucine, tryptophan, serine, histidine and lysine were detected only in 1, 2, or 3 species. Lauric, myristic, palmitoleic, palmitic, heptadecanoic, oleic, stearic, linoleic, arachidic, and behenic were detected in all the investigated *Fusarium* species. However, caprylic, capric, tridecanoic, pentadecanoic, and linolenic were not detected in some of the *Fusarium* species. Among the three determined profiles, the secondary metabolite was the most useful profile, followed by amino acid profile that can be used for chemotaxonomy of the genus *Fusarium*. Many secondary metabolites were limited in distribution among the investigated species. Zearalenone, emodin, β-nitropropionic acid, monorden and wortmanin were detected only in certain species of *Fusarium* and could be considered as taxonomic markers for those species. However, using of a mixed profile of the amino acid, fatty acid and secondary metabolite will led to use more convenient chemotaxonomic markers.

Key words: Secondary metabolites, amino acids, fatty acids, chemotaxonomy, *Fusarium* sp.

INTRODUCTION

Fusarium species have been important for many years as plant pathogens causing diseases such as crown rot, head blight, and scab on cereal grains; vascular wilts on a wide range of horticultural crops; root rots; cankers; and other diseases such as pokkah-boeng on sugarcane and bakanae disease of rice[4]. In the last decades, *Fusarium* species have been studied extensively because the mycotoxins they produce can be a threat to animal and human health[12]. Mycotoxins are secondary metabolites produced by fungi that are associated with a variety of animal diseases and some human health problems[11]. More recently, *Fusarium* species have become important as pathogens of human patients with compromised immune systems[1,2].

Fusarium species are widely distributed in soil and on subterranean and aerial plant parts, plant debris, and other organic substrates. They are common in tropical and temperate regions and are also found in desert, alpine, and arctic areas, where harsh climatic conditions prevail[16]. Many *Fusarium* species are abundant in fertile cultivated and rangeland soils but are relatively uncommon in forest soils[10]. *Fusarium* species are often regarded as soil-borne fungi because of their abundance in soil and their frequent association with plant roots, as either parasites or saprophytes.

The taxonomy of *Fusarium* spp. is confusing and various classification systems have been proposed[14]. Species identification by morphological traits is problematic because characteristics like mycelial pigmentation, formation, shape and size of conidia are unstable and highly dependent on composition of media and environmental conditions. Phenotypic variation is abundant and many expertises are required to distinguish between closely related species and to recognize variation within species[15].

Chemotaxonomy seems to mean different things to different people depending on their subject area. To a bacteriologist, chemotaxonomy has meant nucleotide, amino acid, carbohydrate or lipid based taxonomy[6], and to a mycologist working with yeasts it often means a carbohydrate or lipid-based taxonomy[22,23]. In filamentous fungi, chemotaxonomy was originally seen as a supplement to traditional morphologically based taxonomy[24], whereas it was used early as an important part in both lichen taxonomy[8,9] and basidiomycete taxonomy[3,13].

Chemotaxonomy is traditionally restricted to comprise fatty acids, proteins, carbohydrates, or secondary metabolites, but has sometimes been defined so broadly that it also includes DNA sequences. It is not yet possible to use secondary metabolites in phylogeny, because of the inconsistent distribution

Corresponding Author: Zain, M.E., Medical Laboratory Sciences Department, College of Applied Medical Sciences, Al-Kharj University, KSA.

throughout the fungal kingdom. However, this is the very quality that makes secondary metabolites so useful in classification and identification[7].

An objective of the present study was to determine the role and importance of using amino acids, fatty acids and secondary metabolites as biochemical marker in taxonomy of the genus *Fusarium*.

MATERIALS AND METHODS

Fungal Strains: *Fusarium oxysporum* Schlechtendahl: Fries f. sp. *conglutinans* (Wollenweber) Snyder & Hansen DSMZ 62045, *F. roseum* Link emend. Snyder & Hansen DSMZ 3019, *F. **sacchari*** var. *elongatum* Nirenberg DSMZ 62272, *F. anthophilum* (A. Braun) Wollenweber DSMZ 63270, *F. graminum* Corda DSMZ 62224, and *F. avenaceum* (Corda: Fr.) Saccardo DSMZ 62161 were obtained from the DSMZ - Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (German Collection of Microorganisms and Cell Cultures). Although *Fusarium pseudograminearum* O'Donnell & T. Aoki NRRL 28062, *F. graminearum* Schwabe NRRL 5883, *F. acaciae-mearnsii* Aoki, Kistler, Geiser and O'Donnell NRRL 26752, *F. mesoamericanum* Aoki, Kistler, Geiser and O'Donnell NRRL 25797, and *F. cerealis* (Cooke) Saccardo NRRL 25491 were obtained from Agricultural Research Service Culture Collection (NRRL).

Media: Malt Extract Agar (MEA) (malt extract, 20 g; peptone, 1 g; dextrose, 20 g; agar, 20 g; distilled water, 1 L) was used for cultivation of the fungal strains. The same media (without agar) were used for extraction of the amino acids, fatty acids and secondary metabolites.

Secondary Metabolites: The secondary metabolites were determined according to the method described by Paterson and Bridge[17] as follows:

The fungal growth media, the filtrates, were used for determination of extracellular secondary metabolites. The fungal broth filtrates were extracted with equal volume of chloroform: methanol (2:1, v/v), left to evaporate till dryness, and then dissolved in 3 ml distilled water. The extracts were spotted on a pre-coated Thin Layer Chromatography (TLC) plate (20X20 cm aluminum sheet silica gel 60, layer thickening 0.2 mm) along with griseofulvin as reference standard. The metabolites were eluted using toluene: ethyl acetate: 90 % formic acid (5:4:1, v/v/v) as solvent system. The developed secondary metabolites spots were visualized for their colour and R_f under white, UV (365 nm), UV (254 nm), and return to UV (365 nm), respectively. The plate was then sprayed with 0.5 % *p*-anisaldehyde (in methanol: acetic acid: concentrated sulphuric acid; 17:2:1, v/v/v) and visualized under white light. The plate was heated for 8 minutes at 105°C and visualized under white, UV (365 nm), UV (254 nm) lights, respectively. Identification of secondary metabolites was carried out by comparing colours and R_f values of the obtained spots with the available standard (fusaric acid dissolved in chloroform/methanol 2:1) and with those determined by Paterson and Bridge in (1994).

Fatty Acids: Fungal mycelium was harvested from broth by vacuum filtration. The harvested biomass was rinsed with nanopure water while still in the funnel and then placed on a lipid-free paper towel for several minutes to remove excess moisture.

The lipids were extracted from the dried biomass using chloroform/methanol (2:1 v/v)[5]. One-gram (wet weight) samples of fungal tissue were then placed into 4.0 ml of a saponification reagent and homogenized with a tissue grinder. The homogenate was then reextracted with chloroform/methanol (2:1 v/v).

The extract of each sample was dried under a stream of nitrogen gas, after which the lipid was dissolved in chloroform and methylated. To methylate the liberated fatty acids, 2.0 ml of 54% 6 N HCl in methanol was added to each tube. Subsamples were then placed in an 80°C water bath for 10 min and immediately cooled to room temperature. Prior to FAMEs analyses, the samples were evaporated under nitrogen and resuspended in 50 µl of hexane.

The fatty acid methyl esters were analysed at the Regional Center for Mycology & Biotechnology, Al-Azhar University using a SHIMADZU 5050 gas chromatograph equipped with mass detector using a 30 m x 0.32 mm, 0.53 µm internal diameters, DB1 fused silica capillary column. The carrier gas was helium at a flow rate of 10 ml/min. The temperature of the injector was 250°C and that of the detector was 280°C. The oven temperature after sample injection (2µl) was 1 min at 115°C, increasing to 200°C at 7.5°C/min and then raised at a rate of 5°C per minute to 240°C held at this temperature for 2 min. and then raised at a rate of 3.5°C per minute to 260°C held at this temperature for 2 min.

Peaks were identified by reference to authentic standards and verified using mass selective detector.

Determination of Free Amino Acids and Their Derivatives: The harvested cells were ground with an approximately equal weight of clean cold sand using 70 % ethyl alcohol for the extraction of free amino acids. The obtained slurry was then centrifuged at 6000 rpm for 10 minutes. After centrifugation, the supernatant was decanted to be used for the analysis.

Extraction of Amino Acids: The cell free extract was applied to a small column (0.8 x 12 cm) of dowex 50

(H form) to retain the free amino acids after thoroughly washing with 70 % ethanol. The free amino acids were eluted with 25 ml ammonia in 75 ml of 75 % ethanol. After drying under vacuum in a rotary evaporator, the amino acids were dissolved in 0.2 ml distilled water.

Separation of Amino Acids: Separation of non-protenic amino acids was carried out by using the fully automated Eppendorf / Biotronic (LC 3000) amino acid analyzer at the Regional Center for Mycology and Biotechnology (RCMB) based on ion exchange chromatography followed by post-column detection. The high performance chromatography column (type P) is a cation exchange column for lithium based buffers, the resin particles size used was 4µm and the column size was 125 x 4 mm.

The physiological analysis separation program consisted of a step gradient with 5 buffers and a multislope temperature gradient. The buffers used were Eppendorf / Biotronic type P2.

Amino acids were applied to the separation column by means of an autosampler. They were eluted from the separation column in time sequence by buffers of different pH or ion strength. The buffers were cleared of contaminants by passing through the pre wash column.

Detection was achieved with post column ninhydrin-reagent measured at 570 nm wavelength. The LC 3000 two-channel detector uses 440 nm wavelength for the detection of secondary amino acids. The ninhydrin reagent was mixed with the separated amino acids by second pump, and then the mixture was subjected to elevated temperature in a reactor to form purple chromophore with primary amino acids and a yellow chromophore with secondary amino acids like praline and hydroxyproline. The amount of colored component was measured in a photometer and electronically calculated.

Results: *Fusarium oxysporum* f. sp. *conglutinans*, *F. roseum*, *F. sacchari* var. *elongatum*, *F. anthophilum*, *F. graminum*, and *F. avenaceum*, *F. pseudograminearum*, *F. graminearum*, *F. acaciae-mearnsii*, *F. mesoamericanum*, and *F. cerealis* were cultured on Malt Extract Agar (MEA) (malt extract, 20 g; peptone, 1 g; dextrose, 20 g; agar, 20 g; distilled water, 1 L). The malt extract medium (without agar) was used for extraction of the fungal amino acids, fatty acids and secondary metabolites.

Amino Acid Profiles: The amino acid profiles of the investigated *Fusarium* species were determined (Table 1). The results showed that the most of the amino acids including phosphoserine, taurine, threonine, glycine, tyrosine, phenylalanine, g-amino-n-butyric, carnosine and arginine were detected in all the investigated *Fusarium* species. Although, aspartic acid, citrulline, b-Aminoisobutyric, and 1-Methyl-histidine were detected in 9, out of 11, species.

Interestingly, lysine and histidine were detected in only 3 species, serine were detected in *F. roseum* and *F. mesoa*, leucine was detected only in *F. oxysporum* f. sp. *conglutinans* and tryptophan was detected only in *F. anthophilum* (Table 1). On the other hand, *F. oxysporum* f. sp. *conglutinans* and *F. anthophilum* contained the highest number of amino acids, 21, followed by *F. acaciae-mearnsii*; *F. mesoamericanum* and *F. cerealis* which contained 20 amino acids (Table 1).

Fatty Acid Profiles: The fatty acid profiles of the investigated *Fusarium* species were determined (Table 2). The results showed that the most of the fatty acids including lauric, myristic, palmitic, heptadecanoic, oleic, stearic, linoleic, arachidic, and behenic were detected in all the investigated *Fusarium* species. However, pentadecanoic was detected in all species except *F. anthophilum*, caprylic and linolenic were detected in 9, capric in 8, and tridecanoic was detected in 7 (Table 2).

The highest number of fatty acids, 15, was detected in 5 species; *F. oxysporum* f. sp. *conglutinans*, *F. pseudograminearum*, *F. sacchari* var. *elongatum*, *F. graminum,*and *F. avenaceum*. On the other hand, the lowest number of fatty acids, 11, was detected in *F. anthophilum* (Table 2).

Secondary Metabolite Profiles: The secondary metabolite profiles of 11 *Fusarium* species were determined (Table 3). Secondary metabolites such as aurofusarin, butenolide, diacetoxyscripenol, dihydrofusarubin, ergosterol, fumonisin B, fusaric acid, and zearalenone were detected in many species of *Fusarium*.

On the other hand, emodin was detected only in *F. oxysporum* f. sp. *conglutinans*, β-nitropropionic acid in *F. mesoamericanum*, zearalenol in *F. anthophilum*. Dexynivalenol was detected in booth *F. sacchari* var. *elongatum* and *F. graminearum*, monorden in *F. oxysporum* f. sp. *conglutinans* and *F. pseudograminearum*, moniliformin in *F. roseum*, *F. pseudograminearum*, and *F. mesoamericanum*, and wortmanin in *F. roseum*, *F. sacchari* var. *elongatum*, and *F. avenaceum* (Table 3).

The secondary metabolites dipicolinic acid, fusarone-X, gentisyl alcohol, HT-2 toxin, and neosolaniol were detected in four, out of the 11, *Fusarium* species. Although, T-2 toxin was detected in five species of the investigated *Fusarium* species (Table 3).

Table 1: The amino acid profiles of *Fusarium* species grown on MEA medium. The data are expressed as the percentage of amino acid present.

Amino Acids	*F. oxys* f.sp.*con*	*F. roseum*	*F. pseudog*	*F. sacch v. elong*	*F. anthop*	*F. gramin*	*F. graminea*	*F. acaciae-*	*F. mesoa*	*F. cerealis*	*F. avenac*
Phosphoserine	1.6	5.41	5.17	2.68	2.1	4.86	7.9	0.6	1.18	0.86	3.51
Taurine	2.87	2.97	4.62	0.69	1.83	3.85	2.59	0.14	0.44	0.44	2.75
Phosphoethanolamine	5.2	3.77	5.3	2.93	2.53	3.6	1.15	0.26	0.68	0.38	3.35
Urea	0.73	1.33	4.6	0.96	1.75	2.87	1.65	0.64	0.71	0.95	3.83
Aspartic acid	3.65	4.32	0.15	0	4.17	3.2	0.83	2.88	0	8.02	4.94
Serine	0	0.62	0	0	0	0	0	0	1.23	0	0
Threonine	6.31	7.08	5.44	7.26	16.5	7.5	2.39	14.91	4.01	14.1	7.5
Glutamic acid	29.57	0	11.49	3.23	13.25	0	1.41	15.39	2.23	22.85	0
Glycine	3.16	11.63	6.6	9.82	11.16	12.3	3.38	19.94	10.65	10.01	8.75
Citrulline	0.31	0	0.76	4.96	3.18	1.6	0.7	0	2.9	2.03	3.1
Valine	15.69	6.02	1.16	0	0	9.97	0.41	6.19	0	0	6.82
Cystine	0	0	4.76	0	1.8	0	14.16	2.45	0	2.95	0
Methionine	0	4.25	4.72	2.34	1.68	2.25	7.75	0.26	1.84	0.63	5.05
Leucine	3.27	0	0	0	0	0	0	0	0	0	0
Tyrosine	1.56	2.44	3.67	2.79	4.75	4.55	6.64	2.32	5.29	2.6	3.6
Phenylalanine	1.85	4.26	6.63	3.92	3.25	3.2	9.66	3.27	12.42	4.09	3.1
b-Alanine	2.33	0	0	7.99	6.25	0	0	3.78	7.39	4.91	0
b-Aminoisobutyric	0.88	3.46	0	6.2	4.77	4.7	0	2.64	5.8	2.77	6.68
g-amino-n-butyric	15.64	23.44	11.29	15.35	6.24	21.15	7.29	15.06	18.7	11.24	16.17
Histidine	0.01	0	0	0	3.67	0	0	0	0	2.67	0
1-Methyl-histidine	0.12	4.51	2.26	3.65	0	3.41	2.39	1.62	3.49	0	4.76
Tryptophan	0	0	0	0	1.8	0	0	0	0	0	0
Carnosine	1.04	2.62	2.28	4.06	2.05	2.75	7.93	1.21	3.56	1.7	3.6
Lysine	0	1.32	0	0	0	3.12	0	0	0	0	1.07
Ammonium sulphate	2.08	2.7	7.23	8.22	3.56	2.77	18.32	1.43	6.02	2.41	1.72
Arginine	2.13	7.84	11.87	12.95	3.71	2.35	3.44	5.01	11.45	4.4	11.85

F. oxys f.sp.*con*, *Fusarium oxysporum* f. sp. *conglutinans*; *F. pseudog*, *F. pseudograminearum*; *F. sacch v. elong*, *F. sacchari* var. *elongatum*; *F. anthop*, *F. anthophilum*; *F. gramin*, *F. graminum*; *F. graminea*, *F. graminearum*; *F. acaciae-*, *F. acaciae-mearnsii*; *F. mesoa*, *F. mesoamericanum*; *F. avenac*, *F. avenaceum.*

Table 2: The fatty acid profiles of *Fusarium* species grown on MEA medium. The data are expressed as the percentage of fatty acid present.

Fatty Acids		*F. oxys* f.sp.*con*	*F. roseum*	*F. pseudog*	*F. sacch v. elong*	*F. anthop*	*F. gramin*	*F. graminea*	*F. acaciae-*	*F. mesoa*	*F. cerealis*	*F. avenac*
Caprylic	C8	0.03	0.02	0.04	0.05	0.4	0.05	0.01	0.01	0	0	0.04
Capric	C10	0.02	0.08	0.58	0.16	0	0.02	0.02	0.02	0	0	1.33
Lauric	C12	1.87	1.5	0.51	2.39	0.2	0.04	0.06	0.1	0.13	0.13	1.94
Tridecanoic	C13	0.2	1.74	0.06	0.1	0	0.06	0	0	0	0.02	0.15
Myristic	C14	1.62	0.63	0.89	2.08	0.1	0.08	0.07	0.26	0.08	0.16	2.83
Pentadecanoic	C15	0.32	0.78	0.05	0.1	0	0.02	0.02	0.02	0.02	0.05	0.51
Palmitoleic	C16:1	2.94	1.4	0.76	0.63	0.2	0.24	0.12	0.26	0.1	0.1	1.78
Palmitic	C16	28.9	14.13	26.78	20.31	4.21	13.32	13.3	16.29	17.22	15.5	13.19
Heptadecanoic	C17	6.4	18.82	6.29	24.38	0.54	0.94	2.05	0.8	2.14	0.14	17.45
Oleic	C18:1	19.2	41.6	37.23	18.99	71.6	73.81	75.31	38.2	68.2	74.1	15.44
Stearic	C18	9.43	4.61	14.03	16.67	22.24	7.48	6.28	6.52	10.3	7.84	5.94
Linoleic	C18:2	16.61	14.35	8.21	2.64	0.3	2.86	1.46	23.66	0.96	0.57	17.64
Linolenic	C18:3	11.52	0	3.62	11	0	0.76	0.25	13.26	0.56	0.35	20.85
Arachidic	C20	0.82	0.25	0.67	0.3	0.05	0.27	0.42	0.54	0.22	0.29	0.75
Behenic	C22	0.12	0.09	0.28	0.2	0.16	0.05	0.63	0.06	0.07	0.75	0.16
Total Saturated		49.73	42.65	50.18	66.74	27.9	22.33	22.86	24.62	30.2	24.88	44.29
Total Unsaturated		50.27	57.35	49.82	33.26	72.1	77.67	77.14	75.38	69.8	75.12	55.71

F. oxys f.sp.*con*, *Fusarium oxysporum* f. sp. *conglutinans*; *F. pseudog*, *Fusarium pseudograminearum*; *F. sacch v. elong*, *Fusarium sacchari* var. *elongatum*; *F. anthop*, *Fusarium anthophilum*; *F. gramin*, *Fusarium graminum*; *F. graminea*, *Fusarium graminearum*; *F. acaciae-*, *Fusarium acaciae-mearnsii*; *F. mesoa*, *Fusarium mesoamericanum*; *F. avenac*, *Fusarium avenaceum.*

Table 3: The secondary metabolite profiles of *Fusarium* species grown on MEA medium.

Fatty Acids	*F. oxys* f.sp.*con*	*F. roseum*	*F. pseudog*	*F. sacch v. elong*	*F. anthop*	*F. gramin*	*F. graminea*	*F. acaciae-*	*F. mesoa*	*F. cerealis*	*F. avenac*
Aurofusarin	0	+	+	+	0	0	+	+	0	0	0
Butenolide	+	+	+	+	+	0	+	+	0	0	0
Deoxynivalenol	0	0	0	+	0	0	+	0	0	0	0
Diacetoxyscripenol	+	+	+	0	+	+	+	0	+	+	+
Dihydrofusarubin	+	0	0	+	0	+	0	+	+	+	0
Dipicolinic acid	0	+	0	0	+	0	0	0	+	0	+
Emodin	+	0	0	0	0	0	0	0	0	0	0
Ergosterol	+	0	0	0	+	0	+	+	+	+	+
Fumonisin B	0	+	0	+	+	0	0	0	+	0	0
Fusaric acid	+	0	+	+	+	+	+	0	0	0	0
Fusarone-X	0	0	0	0	0	0	+	0	+	+	+
Gentisyl alcohol	0	+	+	0	0	0	0	0	+	+	0
HT-2 Toxin	0	0	0	+	0	0	+	0	+	0	+
Moniliformin	0	+	+	0	0	0	0	0	+	0	0
Monorden	+	0	+	0	0	0	0	0	0	0	0
Neosolaniol	0	+	0	0	0	+	0	0	0	+	0
β-nitropropionic acid	0	0	0	0	0	0	0	0	+	0	0
Nivalenol	0	0	+	+	+	+	+	0	+	+	+
T-2 Toxin	+	0	+	0	0	+	0	+	0	+	0
Unknown 1	+	+	+	+	+	+	+	+	+	+	+
Unknown 2	0	+	+	0	0	0	0	+	0	0	+
Unknown 3	0	+	0	+	+	+	0	+	+	+	+
Unknown 4	0	0	0	0	0	0	0	0	0	0	+
Unknown 5	0	0	0	0	0	0	0	0	0	0	0
Unknown 6	0	+	0	0	0	+	0	0	0	0	0
Unknown 7	0	0	+	0	0	0	0	+	0	0	0
Unknown 8	0	0	0	0	+	0	0	0	0	0	0
Unknown 9	0	0	0	0	0	0	0	0	0	0	0
Unknown 10	0	0	0	+	0	0	0	0	0	0	0
Unknown 11	0	0	0	+	0	0	0	0	0	0	0
Wortmanin	0	+	0	+	0	0	0	0	0	0	+
Zearalenol	0	0	0	0	+	0	0	0	0	0	0
Zearalenone	+	+	+	+	+	+	0	+	+	+	+

F. oxys f.sp.*con*, *Fusarium oxysporum* f. sp. *conglutinans*; *F. pseudog*, *Fusarium pseudograminearum*; *F. sacch v. elong*, *Fusarium sacchari* var. *elongatum*; *F. anthop*, *Fusarium anthophilum*; *F. gramin*, *Fusarium graminum*; *F. graminea*, *Fusarium graminearum*; *F. acaciae-*, *Fusarium acaciae-mearnsii*; *F. mesoa*, *Fusarium mesoamericanum*; *F. avenac*, *Fusarium avenaceum*.

Discussion: Chemotaxonomy is traditionally restricted to comprise fatty acids, proteins, carbohydrates, or secondary metabolites, but has sometimes been defined so broadly that it also includes DNA sequences[7]. The amino acid, fatty acid, and the secondary metabolite profiles of 11 *Fusarium* species were determined and assessed to be used as a biochemical markers. The results of current study revealed that the secondary metabolite profile was the most effective and informative marker, followed by fatty acid profile, to be applied for taxonomy of the genus *Fusarium*.

Recently, the genus *Fusarium* and associated teleomorphs in *Gibberella* were examined chemotaxonomically and the species-specific profiles of secondary metabolites were shown to exist[18-21]. The current study has confirmed this concept, the obtained results showed that numerous secondary metabolites were restricted in distribution and coined to only one species of *Fusarium*.

The taxonomy of *Fusarium* species has always been a controversial issue[16]. However, The results obtained from this study strongly suggested that introducing a mixed profile concluded from amino acid, fatty acid, and secondary metabolite profiles will be effective and useful in taxonomy of the genus *Fusarium*.

REFERENCES

1. Anaissie E., H. Kantarjian, P. Jones, B. Barlogie, M. Luna, G. Lopez-Berenstein and G. Bodey, 1986. *Fusarium*, a newly recognized fungal pathogen in immunosuppressed patients. Cancer, 57: 2141-2145.
2. Anaissie, E., P. Nelson, M. Beremand, D. Kontoyannis and M. Rinaldi, 1992. *Fusarium* caused hyalohyphomycosis, an overview. Curr. Top. Med. Mycol., 4: 231-249.
3. Benedict R.G., 1970. Chemotaxonomic relationships among basidiomycetes. Advances in Applied Microbiology, 13: 1-23.
4. Booth, C., 1971. The Genus *Fusarium*, p. 237. Commonwealth Mycology Institute, Kew, Surrey, England.
5. Folch, J., M. Lees and G.H. Sloane-Stanley, 1957. A simple method for the isolation and purification of total lipid from animal tissue. J. Biol. Chem., 226: 497-509.
6. Fox A., S.L. Morgan, L. Larsson, G. Odham, (eds), 1990. Analytical Microbiology Methods. Chromatography and Mass Spectrometry. Plenum Press, New York.
7. Frsivad, J.C., B. Andersen and U. Thrane, 2008. The use of secondary metabolite profiling in chemotaxonomy of filamentous fungi. Mycological Research, 112: 231- 240.
8. Hawksworth, D.L., 1973. Some advances in the study of lichens since the time of E.M. Holmes. Botanical Journal of the Linnean Society, 67: 3-31.
9. Hawksworth, D.L., 1976. Lichen chemotaxonomy. In: Brown DH, Hawksworth DL, Bailey RH (eds), Lichenology: progress and problems Academic Press, London, pp: 139-184.
10. Jeschke, N., P.E. Nelson and W.F.O. Marasas, 1990. *Fusarium* species isolated from soil samples collected at different altitudes in the Transkei, southern Africa. Mycologia, 82: 727-733.
11. Marasas, W.F.O. and P.E. Nelson, 1987. Mycotoxicology: introduction to the mycology, plant pathology, chemistry, toxicology and pathology of naturally occurring mycotoxicoses in animals and man. Pennsylvania State University Press, University Park.
12. Marasas, W.F.O., P.E. Nelson and T.A. Toussoun, 1984. Toxigenic *Fusarium* species: identity and mycotoxicology. Pennsylvania State University Press, University Park.
13. Moser, M., 1985. The relevance of chemical characters for the taxonomy of the Agaricales. Proceedings of the Indian Academy of Sciences (Plant Science), 94: 319-339.
14. Nelson, P.E., 1991. History of *Fusarium* systematics. Phytopathology, 81: 1045-1048.
15. Nelson, P.E., T.A. Tossoun and W.F.O. Marasas, 1983. *Fusarium* species, an illustrated manual for identification. The Pennsylvania State University Press, University Park and London.
16. Nelson, P., M.C. Dignan and E. Anaissie, 1994. Taxonomy, Biology and Clinical Aspects of *Fusarium* Species. Clinical Microbiology Reviews, 7(4): 479-504.
17. Paterson, R.R.M. and P.D. Bridge, 1994. Biochemical techniques for filamentous fungi. CAB International, Wallingford, United Kingdom.
18. Schmidt, H., A. Adler, A. Holst-Jensen, S.S. Klemsdal, A. Logrieco, R.L. Mach, H.I. Nirenberg, U. Thrane, M. Torp, R.F. Vogel, T. Yli-Mattila, L. Niessen, 2004. An integrated taxonomic study of *Fusarium langsethiae*, Fusarium poae and *Fusarium sporotrichioides* based on the use of composite datasets. International Journal of Food Microbiology, 95: 341-349.
19. Thrane, U., 1990. Grouping *Fusarium* section Discolor isolates by statistical analysis of quantitative high performance liquid cheromatographic data on secondary metabolite production. Journal of Microbiological Methods, 12: 23-39.
20. Thrane, U., 1993. *Fusarium* species and their specific profiles of secondary metabolites. In: Che1kowski J (ed), *Fusarium*: mycotoxins, taxonomy and pathogenicity. Elsevier, Amsterdam, pp: 199-225.
21. Thrane, U. and U. Hansen, 1995. Chemical and physiological characterization of taxa in the *Fusarium sambucinum* complex. Mycopathologia, 129: 183-190.

22. Tosch, W., K. Lanthaler, V. Boote, D. Stretz, G.D. Robson, E. Geiger, D.B. Drucker, 2006. Molecular species of phosphatidylethanolamine from continuous cultures of *Saccharomyces pastorianus* syn. carlsbergensis strain. Yeast, 23: 75-82.
23. Velazques, E., M. del Villar, I. Grondona, E. Monte, T. Gonzalez-Villa, 2006. Ultrastructural and chemotaxonomic analysis of a xylanolytic strain of *Cryptococcus adeliensis* isolated from sheep droppings in Spain. Archives of Microbiology, 186: 195-202.
24. Tyrrell, D., 1969. Biochemical systematics and fungi. Botanical Review, 35: 305-316.