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ORIGINAL ARTICLE

Effect of chemical compounds on amino acid content of some *Fusarium* species and its significance to fungal chemotaxonomy

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Abstract The cellular amino acid profiles of nine species of *Fusarium*; namely, *Fusarium anthophilum*, *Fusarium avenaceum*, *Fusarium cerealis*, *Fusarium graminearum*, *Fusarium graminum*, *Fusarium oxysporum* f. sp. *conglutinans*, *Fusarium pseudograminearum*, *Fusarium roseum*, and *Fusarium sacchari* var. *elongatum* growing on malt extract medium were determined. The amino acid profiles of the investigated fungi were varied and could be used for identification and characterisation of certain *Fusarium* species. Addition of certain chemical compounds including aspartic acid, glutamic acid, methionine, selenium, and urea to the growth medium affected the amino acid profiles. However, susceptibility of amino acid content to environmental conditions increased the variation of amino acid profiles among all the investigated *Fusarium* species. Some amino acids were only produced when certain chemical compounds were added to the growth medium. Valine was produced by *F. anthophilum* only in the presence of aspartic acid or selenium, while serine was produced in the presence of aspartic acid, glutamic acid, or methionine. Also, cysteine was produced by *F. avenaceum* in the presence of glutamic acid or urea. *F. cerealis* produced tryptophan only in the presence of aspartic acid or urea, while *F. graminearum* produced leucine in glutamic, methionine or urea. Similarly, many different amino acids were produced by each *Fusarium* species only in the presence of certain chemical compounds. The results revealed that the amino acid profiles will be more useful for characterisation and identification of fungi if they are determined under different conditions.

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1. Introduction

The distinctive Kingdom of Fungi attracts the attention of chemists due to a great diversity of polyunsaturated, hydroxy, halogenated, and other unusual acids (Kock and Botha, 1999; Dembitsky and Srebnik, 2002; Dembitsky, 2006; Dembitsky et al., 2010). Arseno and betaine containing compounds have also been found in wild fungi (Kunzler and Eichenberger, 1997; Dembitsky and Levitsky, 2004). Many biologically active enzymes including peroxidases, haloperoxidases and others

have been isolated from different fungi and used in the chemical science and industry (Conesa et al., 2002; Dembitsky, 2003; Sinsabaugh, 2005; Demain et al., 2005; Woesten et al., 2007).

The taxonomy of the fungi is in a state of rapid flux at present, especially due to recent papers based on DNA comparisons, which often overturn the assumptions of the older systems of classification (Hibbett, 2007). Therefore, there is no unique generally accepted system at the higher taxonomic levels and there are constant name changes at every level, from species upwards. Fungal species can also have multiple scientific names depending on its life cycle (Deacon, 2005).

Because numerous fungi grow relatively rapidly in pure culture, it is possible to use physiological and biochemical techniques to identify and classify them (Bridge, 1985; Paterson, 1986; Paterson and Bridge, 1994). These techniques have been successfully used in the study of numerous fungi (de Hoog and Gerrits, 1992; de Hoog and Yurlowa, 1994; de Hoog et al., 1995; Razak et al., 1996, 1997; Yurlowa and de Hoog, 1997; Zain, 2001, 2004; Zain et al., 2009a,b; Kuck and Hoff, 2010; Sumarah et al., 2010; Kjer et al., 2010).

On the other hand, fungi will be affected by all the environmental factors; (chemical and physical). Physical and chemical factors have a pronounced effect on diagnostic characters of fungi. Fungal growth (spore germination, vegetative growth, sporulation) has a specific set of conditions that are optimal. Important conditions in this set are nutrient types and concentrations, light, temperature, oxygen and water availability (i.e., water activity) (Abdel-Ghany and Zain, 2008; Zain, 2009; Zain et al., 2009a,b, 2011; Mattar et al., 2011).

The objectives of this study were (i) to analyse the cellular amino acid profiles of some species of the genus *Fusarium*, (ii) to evaluate the usage of amino acid profiles to differentiate each species, (iii) to determine the susceptibility of the amino acid profiles to the environmental conditions and (iv) its impact on taxonomy of the fungi.

2. Materials and methods

2.1. Fungal strains

The fungal strains were obtained from different culture collections; *Fusarium oxysporum* Schlechtendahl: Fries f. sp. *conglutinans* (Wollenweber) Snyder & Hansen DSMZ 62045, *Fusarium roseum* Link emend. Snyder & Hansen DSMZ 3019, *Fusarium sacchari* var. *elongatum* Nirenberg DSMZ 62272, *Fusarium anthophilum* (A. Braun) Wollenweber DSMZ 63270, *Fusarium graminum* Corda DSMZ 62224, and *Fusarium 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). While *Fusarium pseudograminearum* O'Donnell & T. Aoki NRRL 28062, *Fusarium graminearum* Schwabe NRRL 5883, *Fusarium acaciae-mearnsii* Aoki, Kistler, Geiser and O'Donnell NRRL 26752, *Fusarium mesoamericanum* Aoki, Kistler, Geiser and O'Donnell NRRL 25797, and *Fusarium cerealis* (Cooke) Saccardo NRRL 25491 were obtained from the Agricultural Research Service Culture Collection (NRRL).

2.2. Media

For growth and maintenance of stock cultures, the malt extract agar (MEA) (malt extract, 20 g; peptone, 1 g; glucose, 20; agar, 20 g; and distiled water, 1 l) was used. To determine the effect of chemical compounds, the fungal strains were grown on malt extract broth (MEB) medium separately supplemented with different chemical compounds with the concentration of 0.1% (1 g/l) (aspartic acid, glutamic acid, methionine, and urea) and 0.01% (0.1 g/l) for selenium selenite and incubated at 25 °C for 10 days. Discs, 9 mm in diameter, of agar media containing the fungal materials were picked up from the margin of actively growing colonies, using sterile cork borer and each disc was transferred into 100 ml of liquid medium; in 500 ml conical flasks. The flasks were then incubated at 25 °C for 10 days.

2.3. Determination of the free amino acids and their derivatives

The mycelia mats were harvested and grounded with an approximately equal weight of clean sand using 70% ethyl alcohol for the extraction of free amino acids. The obtained slurry was centrifuged at 6000 rpm for 10 min and the supernatant was decanted to be used for the analysis of amino acids.

2.4. Extraction of amino acids

The cell free extract was applied to a small column (0.8×12 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 distiled water.

2.5. Separation of amino acids

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

The physiological analysis separation programme 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 prewashed column.

Detection was achieved with post column ninhydrin-reagent measured at 570 nm wavelength. The LC 3000 two-channel detector uses 440 nm wavelengths 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 coloured component was measured in a photometer and electronically calculated.

3. Results

The amino acid profile of *F. anthophilum* was affected by the chemical constituents of the growth medium (Table 1). Valine,

Table 1 The amino acid profile of *Fusarium anthophilum* grown on MEB medium amended with different chemical compounds.

Amino acids	Percentage of amino acids in presence of					
	Control	Aspartic acid	Glutamic acid	Methionine	Urea	Selenium
Phosphoserine	02.10	03.17	03.35	03.17	04.32	04.32
Taurine	01.83	04.52	05.70	02.50	03.46	05.49
Phosphoethanolamine	02.53	03.40	00.00	02.28	03.59	04.50
Urea	01.75	00.00	00.00	02.06	03.25	06.32
Aspartic acid	04.17	04.35	04.65	04.88	02.02	00.38
Serine	00.00	04.40	05.79	05. 98	00.00	00.00
Threonine	16.50	04.40	06.89	05.73	05.70	08.27
Glutamic acid	13.25	03.45	00.00	16.52	02.88	04.24
Glycine	11.16	11.64	00.00	00.66	04.83	09.87
Citrulline	03.18	01.80	04.20	04.48	03.40	02.52
Valine	00.00	03.56	00.00	00.00	00.00	02.34
Cystine	01.80	06.36	08.47	04.49	03.63	04.08
Methionine	01.68	03.52	03.35	00.00	03.40	04.95
Leucine	00.00	00.00	03.16	02.67	02.24	00.00
Tyrosine	04.75	02.87	03.17	02.22	00.00	04.44
Phenylalanine	03.25	05.13	06.16	06.38	08.87	04.88
β-Alanine	06.25	00.00	03.13	02.19	03.44	00.00
β-Aminoisobutyric	04.77	10.79	09.11	07.42	04.50	05.25
γ-Amino-*n*-butyric	06.24	04.06	00.00	00.00	04.83	03.40
Histidine	03.67	02.17	06.76	03.38	00.00	00.00
1-Methyl-histidine	00.00	00.00	06.32	02.82	05.55	02.56
Tryptophan	01.80	00.00	03.14	00.00	03.52	00.00
Carnosine	02.05	03.48	03.32	00.58	03.57	02.52
Ammonium sulphate	03.56	04.63	03.47	03.31	04.42	06.13
Arginine	03.71	12.30	10.45	15.29	17.59	13.54

leucine and 1-methyl-histidine were not produced by *F. anthophilum* grown on malt extract medium. On the other hand, β-alanine and tryptophan were not produced in the presence of aspartic acid in the growth medium. Phosphoethanolamine, glutamic acid, glycine, and γ-amino-*n*-butyric were prevented by glutamic acid. Methionine, γ-amino-*n*-butyric and tryptophan were found to be the only amino acids which were prevented by methionine. Tyrosine and histidine were prevented by urea. The presence of selenium prevented the production of β-alanine, histidine and tryptophan (Table 1).

Some amino acids which were not produced by *F. anthophilum* grown on malt extract medium were stimulated for production by different treatments as following: serine induced by aspartic acid, glutamic acid and methionine; valine induced by aspartic acid and selenium; leucine by glutamic acid, methionine, and urea; and 1-methyl-histidine by glutamic acid, methionine, urea, and selenium (Table 1).

The amino acid profile of *F. avenaceum* was affected by the addition of some chemical compounds to the growth medium (Table 2). Most of the amino acids were produced by *F. avenaceum*, however, glutamic acid, cystine, leucine, β-alanine, histidine, and tryptophan were not produced by *F. avenaceum* grown on malt extract medium (Table 2).

The glutamic acid was produced in the presence of all chemicals added to MEB medium (aspartic acid, glutamic acid, methionine, urea, and selenium). While cystine was produced in the presence of glutamic acid and urea (Table 2). Leucine was produced in the presence of glutamic acid and selenium. β-Alanine was produced in the presence of glutamic acid, methionine, urea and selenium (Table 2). Histidine was produced in the presence of aspartic acid, glutamic acid, urea, and selenium. Tryptophan was produced in the presence of urea and selenium. On the other hand, the addition of methionine to the growth medium prevented the production of aspartic acid, valine, and lysine. While the addition of glutamic acid to the medium prevented the production of threonine, valine, and phenylalanine (Table 2).

Most of the amino acids were produced by *F. cerealis*, and approximately more than half the number of amino acids were produced in the presence of aspartic acid, glutamic acid, methionine, urea, and selenium (Table 3). Valine, leucine, 1-methyl-histidine, tryptophan and lysine were not produced by *F. cerealis* grown on malt extract medium. However, the addition of aspartic acid to the growth medium prevented the production of glutamic acid, cystine, phenylalanine, β-alanine and histidine and stimulated the production of valine, 1-methyl-histidine, tryptophan and lysine. On the other hand, glutamic acid prevented the production of aspartic acid, glycine, cystine and histidine and produced leucine (Table 3).

The presence of methionine in the medium prevented the production of cystine, tyrosine and histidine, but produced leucine, 1-methyl-histidine and lysine. Eight amino acids were prevented by urea, while valine, 1-methyl-histidine and tryptophan were produced. Cystine, tyrosine and β-alanine were prevented by selenium, while valine, 1-methyl-histidine and lysine were produced (Table 3). Cystine was not produced in the presence of aspartic acid, glutamic acid, methionine, urea, and selenium (Table 3).

Six amino acids, namely; serine, leucine, β-alanine, β-aminoisobutyric histidine and lysine, were not produced by *F. graminearum* grown on malt extract medium (Table 4). The addition of glutamic acid to the growth medium prevented only one amino acid, valine and induced the production of leucine, β-alanine, β-aminoisobutyric, histidine, and lysine (Table 4).

Table 2 The amino acid profile of *Fusarium avenaceum* grown on MEB medium amended with different chemical compounds.

Amino acids	Percentage of amino acids in presence of					
	Control	Aspartic acid	Glutamic acid	Methionine	Urea	Selenium
Phosphoserine	03.51	03.38	01.66	02.63	02.80	02.42
Taurine	02.75	02.21	01.22	01.60	02.13	01.65
Phosphoethanolamine	03.35	04.90	00.12	01.98	03.33	01.30
Urea	03.83	03.53	01.22	02.05	03.95	02.10
Aspartic acid	04.94	04.51	02.50	00.00	04.57	02.27
Threonine	07.50	09.30	00.00	05.34	13.10	00.00
Glutamic acid	00.00	12.48	14.88	04.14	11.05	03.86
Glycine	08.75	12.24	15.26	07.85	12.26	02.70
Citrulline	03.10	01.50	07.50	04.99	03.28	02.79
Valine	06.82	04.99	00.00	00.00	00.00	03.40
Cystine	00.00	00.00	03.00	00.00	02.80	00.00
Methionine	05.05	03.63	03.10	03.31	02.68	01.63
Leucine	00.00	00.00	01.17	00.00	00.00	02.40
Tyrosine	03.60	00.00	02.10	03.76	02.75	02.55
Phenylalanine	03.10	05.01	00.00	03.95	04.25	00.00
β-Alanine	00.00	00.00	02.18	04.95	05.32	11.55
β-Aminoisobutyric	06.68	04.95	03.45	07.24	05.70	06.40
γ-Amino-*n*-butyric	16.17	06.09	05.68	15.41	05.20	08.61
Histidine	00.00	08.91	18.00	00.00	02.71	14.87
1-Methyl-histidine	04.76	01.45	02.95	05.65	00.00	00.00
Tryptophan	00.00	00.00	00.00	00.00	02.50	05.50
Carnosine	03.60	02.00	03.79	05.00	03.35	08.63
Lysine	01.07	01.66	01.47	00.00	00.00	03.42
Ammonium sulphate	01.72	02.37	02.20	09.37	02.76	02.56
Arginine	11.85	04.90	06.60	10.80	03.51	09.50

Table 3 The amino acid profile of *Fusarium cerealis* grown on MEB medium amended with different chemical compounds.

Amino acids	Percentage of amino acids in presence of					
	Control	Aspartic acid	Glutamic acid	Methionine	Urea	Selenium
Phosphoserine	00.86	02.40	01.86	01.59	05.67	02.38
Taurine	00.44	03.85	01.85	00.54	03.32	03.21
Phosphoethanolamine	00.38	02.36	00.22	01.05	00.00	02.90
Urea	00.95	04.90	00.51	02.02	15.40	05.53
Aspartic acid	08.02	03.87	00.00	00.41	00.00	05.51
Threonine	14.10	08.45	11.80	21.91	04.20	13.30
Glutamic acid	22.85	00.00	07.25	09.14	03.75	09.48
Glycine	10.01	06.70	00.00	09.90	01.40	17.24
Citrulline	02.03	05.20	00.79	01.94	00.00	00.50
Valine	00.00	07.62	00.00	00.00	01.17	03.99
Cystine	02.95	00.00	00.00	00.00	00.00	00.00
Methionine	00.63	04.25	00.73	00.61	05.07	02.63
Leucine	00.00	00.00	00.78	04.98	00.00	00.00
Tyrosine	02.60	02.55	02.95	00.00	00.00	00.00
Phenylalanine	04.09	00.00	05.86	05.60	00.00	04.01
β-Alanine	04.91	00.00	14.85	09.44	19.70	00.00
β-Aminoisobutyric	02.77	08.65	09.82	05.87	16.17	03.95
γ-Amino-*n*-butyric	11.24	14.20	19.22	04.72	00.00	05.09
Histidine	02.67	00.00	00.00	00.00	00.00	07.91
1-Methyl-histidine	00.00	05.60	03.52	02.70	02.59	03.45
Tryptophan	00.00	02.15	00.00	00.00	00.84	00.00
Carnosine	01.70	04.76	03.79	02.60	10.03	01.99
Lysine	00.00	02.19	01.30	00.21	00.00	00.67
Ammonium sulphate	02.41	02.65	01.52	01.98	07.79	01.30
Arginine	04.40	09.80	11.37	12.80	02.90	04.97

On the other hand, aspartic acid prevented the production of taurine and valine and induced the production of β-alanine, β-aminoisobutyric and lysine. Interestingly, the addition of methionine to the growth medium induced the production of all amino acids that were not produced by *F. graminearum* grown on malt extract medium (serine, leucine, β-alanine, β-

Table 4 The amino acid profile of *Fusarium graminearum* grown on MEB medium amended with different chemical compounds.

Amino acids	Percentage of amino acids in presence of					
	Control	Aspartic acid	Glutamic acid	Methionine	Urea	Selenium
Phosphoserine	07.90	01.16	07.70	01.42	02.42	05.50
Taurine	02.59	00.00	02.17	02.67	02.56	06.53
Phosphoethanolamine	01.15	01.43	00.27	01.94	00.39	02.44
Urea	01.65	00.46	00.43	01.10	01.15	01.10
Aspartic acid	00.83	00.99	00.53	05.13	01.22	00.00
Serine	00.00	00.00	00.00	02.90	00.00	00.00
Threonine	02.39	06.23	05.88	15.35	05.78	01.18
Glutamic acid	01.41	00.85	01.62	00.00	01.78	00.75
Glycine	03.38	07.35	02.78	00.37	04.75	05.05
Citrulline	00.70	02.39	03.62	14.82	02.44	00.70
Valine	00.41	00.00	00.00	01.08	00.00	00.00
Cystine	14.16	08.38	07.97	22.00	03.59	08.82
Methionine	07.75	04.27	01.67	11.53	01.44	04.52
Leucine	00.00	00.00	01.35	01.86	01.20	00.00
Tyrosine	06.64	05.41	05.61	02.22	00.00	02.63
Phenylalanine	09.66	09.77	07.12	03.60	10.80	05.04
β-Alanine	00.00	09.50	09.14	02.26	08.47	07.16
β-Aminoisobutyric	00.00	08.50	05.58	02.23	07.54	06.42
γ-Amino-*n*-butyric	07.29	07.23	07.35	00.00	23.85	08.56
Histidine	00.00	00.00	01.68	01.63	00.00	08.11
1-Methyl-histidine	02.39	10.22	04.98	00.00	05.52	13.78
Carnosine	07.93	04.21	03.37	01.88	03.53	03.47
Lysine	00.00	01.36	06.41	00.82	01.52	00.00
Ammonium sulphate	18.32	06.87	02.80	01.71	01.49	02.09
Arginine	03.44	03.42	09.97	01.46	08.57	06.15

aminoisobutyric, histidine and lysine), while glutamic acid, γ-amino-*n*-butyric and 1-methyl-histidine were prevented by methionine (Table 4).

The amino acid profile of *F. graminum* was affected by the chemical constituents of the growth medium (Table 5). Three amino acids; glutamic acid, leucine, and β-alanine were not detected in the extract of *F. graminum* grown on malt broth medium. However, treatment by aspartic acid, glutamic acid, methionine, urea and selenium encouraged the production of glutamic acid. While leucine amino acid was produced by aspartic acid and selenium, methionine and urea prevented the production of β-alanine (Table 5).

Table 5 The amino acid profile of *Fusarium graminum* grown on MEB medium amended with different chemical compounds.

Amino acids	Percentage of amino acids in presence of					
	Control	Aspartic acid	Glutamic acid	Methionine	Urea	Selenium
Phosphoserine	04.86	03.87	04.74	05.65	02.59	04.51
Taurine	03.85	02.86	03.91	03.96	02.97	03.60
Phosphoethanolamine	03.60	04.65	03.66	04.77	03.75	03.85
Urea	02.87	03.80	02.51	03.80	04.60	02.72
Aspartic acid	03.20	07.17	03.32	03.25	04.35	03.35
Threonine	07.50	05.20	07.62	04.55	06.62	07.75
Glutamic acid	00.00	10.75	18.27	13.05	16.42	05.25
Glycine	12.30	09.50	02.70	03.52	06.50	09.42
Citrulline	01.60	03.55	02.67	02.75	02.33	00.00
Valine	09.97	02.60	02.75	00.00	03.87	03.90
Methionine	02.25	05.75	04.30	03.30	04.40	04.40
Leucine	00.00	04.25	00.00	00.00	00.00	03.65
Tyrosine	04.55	00.00	04.40	06.60	02.28	05.20
Phenylalanine	03.20	04.25	03.35	04.05	04.32	05.45
β-Alanine	00.00	00.00	00.00	04.70	05.50	00.00
β-Aminoisobutyric	04.70	04.55	04.45	05.85	05.43	04.75
γ-Amino-*n*-butyric	21.15	04.45	02.35	03.37	00.00	04.40
1-Methyl-histidine	03.41	00.00	03.60	03.46	04.53	05.06
Carnosine	02.75	04.25	00.00	00.00	00.00	00.00
Lysine	03.12	05.28	03.03	03.10	05.27	06.47
Ammonium sulphate	02.77	03.37	12.40	13.52	04.57	06.65
Arginine	02.35	09.90	09.97	06.75	09.70	09.62

Tyrosine and 1-methyl-histidine were absent in the presence of aspartic acid. Only carnosine was absent in the presence of glutamic acid. Valine and carnosine did not show in the presence of methionine, γ-amino-*n*-butyric and carnosine disappeared in the presence of urea, finally citrulline, β-alanine and carnosine did not produce in the presence of selenium (Table 5).

The amino acids produced by *F. oxysporum* f. sp. *conglutinans* including phosphoserine, taurine, threonine, glutamic acid, valine, phenylalanine, β-alanine, β-aminoisobutyric, 1-methyl-histidine, carnosine, and arginine were also produced when aspartic acid, glutamic acid, methionine, urea, and selenium were separately added to the growth medium (Table 6).

Cystine, methionine, tryptophan, and lysine were not produced by *F. oxysporum* f. sp. *conglutinans* grown on malt broth medium; however, cystine was produced when aspartic acid, glutamic acid, methionine, urea, and selenium were added separately to the growth medium. Also, methionine was produced in the presence of aspartic acid, glutamic acid, urea, and selenium. Lysine was produced in the presence of methionine and urea, while tryptophan was produced only in the presence of urea (Table 6).

Glutamic acid had different effects on the amino acid profile of *F. pseudograminearum*, it prevented the production of carnosine while induced the production of serine and lysine. The addition of methionine to the growth medium induced the production of serine, leucine, β-alanine and histidine. Methionine prevented the production of each of phosphoethanolamine, citrulline, valine and γ-amino-*n*-butyric (Table 7).

Two amino acids, cystine and carnosine, were prevented while lysine was produced in the presence of urea. Selenium induced the production of histidine and prevented 1-methyl-histidine. Additionally, the presence of aspartic acid prevented the production of phosphoethanolamine and citrulline while serine and β-alanine were not produced (Table 7).

The amino acid profile of *F. roseum* was affected by the chemical constituents of the growth medium (Table 8). The amino acids phosphoserine, taurine, aspartic acid, threonine, glycine, valine, methionine, tyrosine, phenylalanine, β-aminoisobutyric, γ-amino-*n*-butyric, 1-methyl-histidine, carnosine, lysine and arginine were produced by *F. roseum* grown on malt extract medium.

However, some amino acids were produced by *F. roseum* only when one or more of the chemical compounds were separately added to the growth medium. Glutamic acid was produced only in the presence of aspartic acid, glutamic acid, urea, and selenium (Table 8). Although, citrulline was produced in the presence of glutamic acid, methionine, and urea; cystine was only produced in the presence of methionine and histidine in the presence of selenium; and leucine in the presence of aspartic acid and urea (Table 8).

On the other hand, glycine was not produced by *F. roseum* when aspartic acid and urea were added to the growth medium; valine was not produced in the presence of methionine and urea; tyrosine β-aminoisobutyric and lysine were not produced in the presence of methionine and selenium (Table 8).

The amino acid profile of *F. roseum* was affected by the chemical constituents of the growth medium (Table 8). The amino acids phosphoserine, taurine, aspartic acid, threonine, glycine, valine, methionine, tyrosine, phenylalanine, β-aminoisobutyric, γ-amino-*n*-butyric, 1-methyl-histidine, carnosine, lysine and arginine were produced by *F. roseum* grown on malt extract medium.

Table 6 The amino acid profile of *Fusarium oxysporum* f. sp. *conglutinans* grown on MEB medium amended with different chemical compounds.

Amino acids	Percentage of amino acids in presence of					
	Control	Aspartic acid	Glutamic acid	Methionine	Urea	Selenium
Phosphoserine	01.60	02.68	02.76	01.92	03.75	05.56
Taurine	02.87	00.27	04.31	02.17	04.24	04.61
Phosphoethanolamine	05.20	01.93	03.55	02.44	00.14	03.76
Urea	00.73	00.21	01.73	00.60	00.94	02.61
Aspartic acid	03.65	00.00	24.15	05.63	01.63	14.50
Threonine	06.31	11.07	09.15	02.40	06.47	11.36
Glutamic acid	29.57	19.19	21.84	47.35	12.87	05.82
Glycine	03.16	03.47	02.17	00.00	03.92	10.15
Citrulline	00.31	00.77	00.27	00.37	00.70	00.00
Valine	15.69	19.48	01.13	14.82	10.99	10.70
Cystine	00.00	02.20	09.64	01.08	03.54	01.56
Methionine	00.00	00.86	06.46	00.00	01.13	01.35
Leucine	03.27	00.64	00.00	06.53	00.28	00.00
Tyrosine	01.56	01.56	00.00	00.86	01.68	01.54
Phenylalanine	01.85	01.85	01.06	01.22	02.19	02.20
β-Alanine	02.33	03.83	02.26	02.60	03.78	04.84
β-Aminoisobutyric	00.88	02.16	00.78	01.26	02.07	02.32
γ-Amino-*n*-butyric	15.64	16.19	02.42	01.23	12.46	03.34
Histidine	00.01	00.68	01.30	00.00	00.91	06.37
1-Methyl-histidine	00.12	01.63	01.29	00.63	00.76	02.66
Tryptophan	00.00	00.00	00.00	00.00	00.83	00.00
Carnosine	01.04	01.74	01.26	00.98	00.56	00.69
Lysine	00.00	00.00	00.00	00.72	17.64	00.00
Ammonium sulphate	02.08	04.36	01.33	02.71	04.09	02.75
Arginine	02.13	03.23	01.16	02.46	02.43	01.32

Table 7 The amino acid profile of *Fusarium pseudograminearum* grown on MEB medium amended with different chemical compounds.

Amino acids	Percentage of amino acids in presence of					
	Control	Aspartic acid	Glutamic acid	Methionine	Urea	Selenium
Phosphoserine	05.17	02.45	03.17	06.05	01.57	04.39
Taurine	04.62	07.34	04.52	03.74	05.24	05.40
Phosphoethanolamine	05.30	00.00	03.40	00.00	08.08	04.52
Urea	04.60	01.25	00.00	02.48	01.72	05.38
Aspartic acid	00.15	03.50	04.35	02.27	03.13	00.37
Serine	00.00	02.24	04.40	02.15	00.00	00.00
Threonine	05.44	08.50	04.40	07.59	06.53	08.22
Glutamic acid	11.49	05.35	03.45	06.34	09.30	04.27
Glycine	06.60	09.74	11.64	07.75	04.83	09.82
Citrulline	00.76	00.00	01.80	00.00	03.13	01.54
Valine	01.16	04.57	03.56	00.00	02.78	02.38
Cystine	04.76	01.35	06.36	06.61	00.00	05.54
Methionine	04.72	07.60	03.52	05.87	03.50	02.94
Leucine	00.00	00.00	00.00	02.01	00.00	00.00
Tyrosine	03.67	00.79	02.87	04.82	04.86	04.45
Phenylalanine	06.63	04.60	05.13	11.48	07.54	04.85
β-Alanine	00.00	05.79	00.00	02.91	04.87	00.00
γ-Amino-*n*-butyric	11.29	07.35	10.79	00.00	09.18	10.28
Histidine	00.00	00.00	00.00	08.54	00.00	03.48
1-Methyl-histidine	02.26	04.20	05.06	03.41	04.37	00.00
Carnosine	02.28	07.22	00.00	04.15	00.00	02.50
Lysine	00.00	00.00	02.48	00.00	06.37	00.00
Ammonium sulphate	07.23	08.28	04.63	07.38	06.35	06.01
Arginine	11.87	08.88	14.47	04.45	06.65	12.66

Table 8 The amino acid profile of *Fusarium roseum* grown on MEB medium amended with different chemical compounds.

Amino acids	Percentage of amino acids in presence of					
	Control	Aspartic acid	Glutamic acid	Methionine	Urea	Selenium
Phosphoserine	05.41	03.03	04.50	05.76	02.65	04.80
Taurine	02.97	02.67	01.17	19.01	04.99	03.50
Phosphoethanolamine	03.77	00.00	00.88	00.00	00.67	07.44
Urea	01.33	00.02	01.96	00.00	00.06	02.45
Aspartic acid	04.94	03.51	03.28	00.63	00.55	00.28
Threonine	07.08	05.80	05.08	00.00	00.00	01.42
Glutamic acid	00.00	12.76	05.63	00.00	00.66	01.24
Glycine	11.63	00.00	32.72	05.86	00.00	04.26
Citrulline	00.00	00.00	00.56	01.11	00.78	00.00
Valine	06.02	01.45	03.68	00.00	00.00	01.60
Cystine	00.00	00.00	00.00	09.77	00.00	00.00
Methionine	04.25	08.22	04.49	14.85	01.32	02.32
Leucine	00.00	02.52	00.00	00.00	01.40	00.00
Tyrosine	02.44	02.09	01.47	00.00	02.06	00.00
Phenylalanine	04.26	03.07	02.32	05.83	02.50	08.90
β-Alanine	00.00	06.25	05.28	14.89	12.96	00.00
β-Aminoisobutyric	03.46	03.04	02.49	00.00	02.08	00.00
γ-Amino-*n*-butyric	23.44	09.34	13.22	03.61	26.63	02.23
Histidine	00.00	00.00	00.00	00.00	00.00	26.48
1-Methyl-histidine	04.51	08.03	03.18	06.57	02.52	15.02
Carnosine	02.62	06.44	01.52	08.22	01.15	04.27
Lysine	01.32	03.02	00.38	00.00	12.56	00.00
Ammonium sulphate	02.70	02.05	01.81	01.44	04.54	02.13
Arginine	07.84	16.69	04.39	02.46	19.92	12.11

However, some amino acids were produced by *F. roseum* only when one or more of the chemical compounds were separately added to the growth medium. Glutamic acid was produced only in the presence of aspartic acid, glutamic acid, urea, and selenium (Table 8). Although, citrulline was produced in the presence of glutamic acid, methionine, and urea; cystine

Table 9 The amino acid profile of *Fusarium sacchari* var. *elongatum* grown on MEB medium amended with different chemical compounds.

Amino acids	Percentage of amino acids in presence of					
	Control	Aspartic acid	Glutamic acid	Methionine	Urea	Selenium
Phosphoserine	02.68	00.40	01.55	02.20	03.76	01.25
Taurine	00.69	02.97	02.82	01.17	02.61	01.72
Phosphoethanolamine	02.93	01.70	01.90	02.00	01.80	00.00
Urea	00.96	01.99	03.99	01.89	02.09	00.81
Aspartic acid	00.00	00.00	00.00	00.00	00.00	04.98
Threonine	07.26	05.40	04.06	03.19	04.00	05.51
Glutamic acid	03.23	05.29	06.43	05.30	07.49	05.28
Glycine	09.82	00.00	06.80	02.80	05.75	07.90
Citrulline	04.96	05.62	03.98	00.00	02.03	03.88
Valine	00.00	06.86	00.00	06.98	00.00	00.00
Cystine	00.00	00.00	00.00	02.27	00.00	00.00
Methionine	02.34	06.60	04.31	00.00	03.20	02.39
Leucine	00.00	00.00	02.95	00.00	00.00	00.00
Tyrosine	02.79	00.00	05.60	04.86	00.00	03.84
Phenylalanine	03.92	07.96	00.00	05.31	06.96	04.80
β-Alanine	07.99	05.73	04.85	09.77	07.90	06.96
β-Aminoisobutyric	06.20	08.46	11.39	07.81	05.30	08.30
γ-Amino-*n*-butyric	15.35	09.20	10.49	11.57	07.44	11.50
Histidine	00.00	00.00	00.00	00.00	05.70	00.00
1-Methyl-histidine	03.65	02.80	05.40	06.10	05.74	05.60
Tryptophan	00.00	00.00	00.00	00.00	00.00	07.15
Carnosine	04.06	02.15	02.41	05.61	06.26	00.00
Lysine	00.00	05.70	00.00	00.00	00.00	00.00
Ammonium sulphate	08.22	13.37	11.45	09.54	11.15	09.20
Arginine	12.95	07.80	09.73	11.64	10.82	08.93

was only produced in the presence of methionine and histidine in the presence of selenium; and leucine in the presence of aspartic acid and urea (Table 8).

On the other hand, glycine was not produced by *F. roseum* when aspartic acid and urea were added to the growth medium; valine was not produced in the presence of methionine and urea; tyrosine β-aminoisobutyric and lysine were not produced in the presence of methionine and selenium (Table 8).

The amino acid profile of *F. sacchari* var. *elongatum* was affected by the chemical constituents of the growth medium (Table 9). Seven amino acids, aspartic acid, valine, cystine, leucine, histidine, tryptophan and lysine, were not produced by *F. sacchari* var. *elongatum* grown on malt extract medium.

However, aspartic acid and tryptophan were only produced in the presence of selenium. Valine and lysine were induced by aspartic acid, also valine and cystine were induced by methionine (Table 9). Only glutamic acid induced formation of leucine amino acid. Urea was the only treatment which induced histidine production. The amino acids prevented from production in the presence of methionine were citrulline and methionine. Tyrosine was prevented from the formation by each of aspartic acid and urea, while phenylalanine and carnosine were prevented by glutamic acid and urea, respectively (Table 9).

4. Discussion

The fungal systematic is still based mainly on morphological criteria and observable characteristics. However, numerous alternative approaches have been developed (Whalley and Edwards, 1987, 1995; Stadler and Hellwig, 2004, 2005; Quang et al., 2005, 2006; Stadler et al., 2005, 2006; Guo et al., 2009; Xia et al., 2009; Zain et al., 2009a,b; Singh et al., 2010; Kjer et al., 2010; Coleman et al., 2011; Qiao et al., 2011; Zhang et al., 2011). It was suggested that the study of the metabolic profiles of sugar and amino acid assimilation in the different isolates could be a complementary tool to classify the different isolates in the *Gibberella fujikuroi* complex. It can be used for this purpose together with the study of the production of secondary metabolites (Leslie et al., 1992; Jimenez et al., 1997) and DNA-based techniques (Mule et al., 1997; Nirenberg and O'Donnell, 1998; Jimenez et al., 2000).

The results of the current study revealed that the amino acids of nine species of *Fusarium* were varied and can be used as complementary markers in fungal taxonomy. On the other hand, the results also revealed that the amino acids were susceptible to the presence of some chemical compounds. Nevertheless, the results obtained from the current study confirmed the validity of using the amino acid profiles as complementary markers in fungal taxonomy. Moreover, some amino acids were only produced in the presence of certain chemical compounds, which may then be used as taxonomic markers under such condition(s).

Referring to the results obtained from the current study, we strongly suggest that the determination of amino acid profiles for identification and characterisation of fungi should be carried out using different growth conditions and must be coined to such conditions.

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