

Studies on amino acid composition of six species of *Drechslera*

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Amino acid composition of six different species of *Drechslera* and their isolates viz. *D. australiensis*, *D. halodes*, *D. spicifera*, *D. ravenellii*, *D. maydis* and *D. dematioidea* were grown in glucose peptone broth and studied using thin layer chromatography. The frequency of occurrence of certain amino acids and their ratios are noteworthy. Alanine, tyrosine, hydroxyproline and isoleucine were absent among all species of *Drechslera*. However, the ratios of free and bound amino acids were different among all the species of *Drechslera* studied.

Key words : *Drechslera* spp., chemotaxonomy, amino acids

INTRODUCTION

Similarity and dissimilarity among protein and amino acid band pattern are the taxonomic markers at species level in fungi (Razak *et al.*, 1997). These are varied at intergeneric level than at interspecific level in different groups of fungi (Parmar *et al.*, 1984). Presence or absence of certain amino acids and their ratios in a particular species of fungi are good subsidiary characters for distinguishing one genera from another (Agarwal, 1980). Ratio of free amino acid to bound form show a great similarity in trend depending on the close relationship of species (Mathur and Sarbhoy, 1977 ; Yamaguchi and Uchida, 1990). At generic level the ratios differ drastically (Sarbhoy, 1963 ; Parmar *et al.*, 1984 ; Iyer *et al.*, 2001). It is intended, therefore, through this study to find the similarities or differences in amino acid pattern and establish its role in chemotaxonomy.

MATERIALS AND METHODS

Cultures of six species of *Drechslera* viz., *D. halodes* (H.C.I.O 42658, 42662), *D. maydis* (ITCC 3294) *D. australiensis* (H.C.I.O 42665, 42663, 42598, 42664), *D. dematioidea* (H.C.I.O 42660), *D. spicifera* (H.C.I.O 42661) and *D. ravenellii* (H.C.I.O. 42659) were used in this study. The fungi were grown in 150 ml Erlenmeyer flasks containing

20 ml of Glucose Peptone Broth. In a separate study it was found that Glucose and Peptone supported the maximum growth among all the species of *Drechslera* studied. (Agarwal *et al.*, 2000).

Three replicates were made in each case. The flasks were inoculated with actively growing mycelial agar disc of above mentioned isolates of *Drechslera* using cork borer. Inoculated flasks were incubated at $25 \pm 2^\circ\text{C}$ for 14 days and mycelium was harvested on the 15th day. The mycelial mats were dried in an electric oven at 25°C for three days and weighed. Free amino acids were obtained using known quantity of mycelium and extracted in 80% Ethyl alcohol. Bound (Protein) amino acids were extracted using 6N HCl (Sadasivam and Manickam, 1993). Standard amino acids were run simultaneously to compare the unknown amino acids of the fungal isolates. The solvent system used was n-butanol : acetic acid : water (4:1:5, v/v). The chromatogram was developed using 0.1% ninhydrin spray. The R_f value was calculated and compared with the standard amino acid. For quantitative analysis method as given by Parmar *et al.* (1984) was used.

RESULTS AND DISCUSSION

Qualitative and quantitative analysis of amino acids

Table 1 : Qualitative and quantitative analysis of amino acids in *Drechslera* sp. ($\mu\text{g/g}$)

Amino Acids	<i>Drechslera maydis</i> (ITCC 3294)		<i>Drechslera australiensis</i> (HCIO 42663)		<i>Drechslera dematioidea</i> (HCIO 42660)		<i>Drechslera australiensis</i> (HCIO 42665)		<i>Drechslera spicifera</i> (HCIO 42661)	
	Free	Bound	Free	Bound	Free	Bound	Free	Bound	Free	Bound
Arginine	—	—	—	—	—	—	—	—	—	—
Glutamic acid	—	—	—	—	—	—	—	—	190	—
Threonine	—	5	—	—	—	—	—	—	—	—
Serine	—	—	—	—	—	—	—	50	—	—
Valine	6	—	140	—	110	21	—	—	—	—
Phenylalanine	—	—	—	—	—	—	—	—	—	6
Leucine	8	20	500	10	—	22	99	13	120	7
Alanine	—	—	—	—	—	—	—	—	—	—
Methionine	—	—	—	—	—	6	97	—	—	9
Histidine	—	—	—	—	—	—	—	—	—	—
Asparagine	20	—	100	—	360	—	—	—	—	—
Hydroxyproline	—	—	—	—	—	—	—	—	—	—
Tyrosine	—	—	—	—	—	—	—	—	—	—
Proline	—	12	—	20	—	20	—	6	—	10.5
Glutamine	—	—	—	10	—	—	—	18	90	—
Lysine	10	—	20	—	—	—	—	—	—	—
Cystine	—	10	—	—	—	—	—	—	140	—
Isoleucine	—	—	—	—	—	—	—	—	—	—
Total	44	47	760	40	470	69	196	87	540	32.5
Ratio (Free : Bound)	1 : 93		19 : 1		6.81 : 1		3.81 : 1		16.6 : 1	

Table 2 : Qualitative and quantitative analysis of amino acids in *Drechslera* species ($\mu\text{g/g}$)

Amino Acids	<i>Drechslera maydis</i> (HCIO 42659)		<i>Drechslera australiensis</i> (HCIO 42658)		<i>Drechslera dematioidea</i> (HCIO 42598)		<i>Drechslera australiensis</i> (HCIO 42664)		<i>Drechslera spicifera</i> (HCIO 42662)	
	Free	Bound	Free	Bound	Free	Bound	Free	Bound	Free	Bound
Arginine	10	—	3.5	—	175	—	—	—	—	—
Glutamic acid	3	—	8	—	—	—	4.0	—	8.0	—
Threonine	—	36	—	—	—	—	—	—	—	—
Serine	—	20	—	—	—	—	—	—	—	—
Valine	—	18	—	50	—	—	—	46	—	—
Phenylalanine	—	—	—	—	—	—	—	—	—	80
Leucine	10	8	11	14	180	50	10	18	11	75
Alanine	—	—	—	—	—	—	—	—	—	—
Methionine	—	—	—	—	—	—	—	—	—	—
Histidine	—	—	—	—	100	—	—	—	—	—
Asparagine	—	—	—	—	—	—	—	—	—	—
Hydroxyproline	—	—	—	—	—	—	—	—	—	—
Tyrosine	—	—	—	—	—	—	—	—	—	—
Proline	—	—	—	20	—	16	—	3.6	—	110
Glutamine	—	—	—	16	—	22	8.5	40	.80	10
Lysine	8	—	0.08	—	—	20	—	—	—	—
Cystine	—	—	—	—	—	—	50	—	10	21
Isoleucine	—	—	—	—	—	—	—	—	—	—
Total	31	82	22.58	100	455	108	72.5	107.6	29.80	296
Ratio (Free : Bound)	1 : 0.268		0.225 : 1		4.1 : 1		1 : 0.517		1 : 10.8	

of all the species of *Drechslera* showed that isoleucine, tyrosine, alanine and hydroxyproline were absent in free and bound form in all the isolates. The ratio of free to bound amino acids were dissimilar among different species but similar among isolates of same species. *D. australiensis* and *D. dematioidea* showed very high ratios of free and bound amino acids. Morphological studies indicate close relationship among these species. (Tables 1 and 2).

Kushwaha (1984) reported different types and quantity of amino acids in their culture filtrate and mycelial extracts among two species of *Microsporium* and *Chrysosporium tropicum*. In a similar study, amino acid composition of four species of *Fusarium* were conducted. Aspartic acid, glycine, alanine, valine, isoleucine, glutamine and phenylalanine were present both in free and bound form. Among bound amino acids, hydroxyproline was detected in all the species of *Fusarium*, proline in *F. solani* and *F. equiseti* only. Serine, leucine, and lysine which were absent in *F. solani* and *F. equiseti* in free form were present in bound form. In spite of some common amino acids, there were ample differences in the amino acid composition enabling the distinction of species (Agarwal and Sarbhoy, 1976).

Mathur and Sarbhoy (1977) also studied the amino acid composition of *Sclerotium rolfsii* isolates causing root rot of sugarbeet and compared it with other isolates of *S. rolfsii* from brinjal, groundnut and larkspur. Glycine and asparagine were present in all the isolates irrespective of the host from which isolated although number of amino acids in free form varied. Soluble culture extracts of *B. (C. hawaiiensis)*, *C. spicifera*, *Exerohilum longirostratum*, *E. meginnisii*, *E. rostratum* (*Setosphaeria rostrata*) and *Helminthosporium solani* were used to prepare reference antisera in New Zealand white rabbits by Pasarell *et al.* (1990). These antisera were tested by a microimmunodiffusion method against concentrated culture filtrates of 115 isolates belonging to *Drechslera* spp., *Bipolaris* spp., *Exerohilum* spp., *Helminthosporium* sp. Cross reactivity were observed among isolates of *Cochliobolus australiensis*, *C. hawaiiensis*, *C. spicifer*, *E. longirostratum*, and *Setosphaeria*

rostrata against respective genera. On the basis of these tests, Pasarell *et al.* (1990) concluded that exoantigen test is a valuable chemotaxonomic method to delineate species of *Drechslera* at generic level. Above experiments indicate common amino acid (protein) patterns among different species of same genera. The present studies done by us indicate common amino acid pattern among different species of the same genera although ratios varied for each species. Razak *et al.* (1997) studied the role of proteins and amino acids at specific level of genus *Aspergillus*. On the basis of study conducted, *A. clavato-nanica* was assigned to *A. clavatus* group and *A. satilli* to an unknown group.

Iyer and Rao (2001) have observed differences in amino acid pattern and also their ratios among the three keratinophilic fungi, viz., *Microsporium gypseum*, *Chrysosporium tropicum* and *Trichophyton mentagrophytes*. These findings were substantiated by cultural and morphological studies.

Our studies clearly indicate that different species of the same genus *Drechslera* show variable ratios of bound and free amino acids depending on the degree of morphological differences. Morphological studies indicate proximity in *D. australiensis* and *D. dematioidea*, in consonance with these studies, their ratios are also similar. (Tables 1 and 2). Therefore, on the basis of these studies it may be concluded that amino acid pattern studies may be used as one of the factors in chemotaxonomic studies to delineate species and genera.

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