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Amino Acids, Sugars and Organic Acids Composition of the Mycelium of Five Species of *Aplosporella* Speg

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Abstract

To identify plant constituents one has to isolate and not only to purify but also to determine the class of compound. The class of compound is normally clear from its response to color tests, its solubility, RF property etc. In addition to chromatographic methods, one methods like UV, IR, NMR and MS measurements are also in practice. But for all practical purposes, the writer has preferred chromatographic methods to analyze different constituents in Aplosporella. The precise mode of extraction depends on the texture and water content of the plant material being extracted and the type of substance that is being isolated.

Keywords: Amino Acids; Sugars; Organic Acids; *Aplosporella*

Introduction

Amino Acids

Amino acids are the skeletal units of proteins and some essential metabolites. Various workers have studied amino acid composition of a number of fungi both in free state as well as in bound state. Fagan M, et al. [1] Venkataraman CS, et al. [2] and Crossan DF, et al. [3] made an attempt to correlate amino acids composition of hyphae with taxonomic position of a fungal species or a group of organisms, while Bilgrami KS, et al. [4] Reddy SM, et al. [5] and Shreemali JL, et al. [6] tried to correlate amino acid composition with pathogenesity of fungal organisms, Agnihotri VP, et al. [7] Vijay Kumar CSK, et al. [8] Jamaluddin S, et al. [9] Mathur SB, et al. [10] and Misra PS, et al [11], have also studied amino acid composition of fungal hyphae and that of spores of some members. Vyawahare SV, et al. [12] have studied to determine the presence of amino acids in the three species of Drechslera in free as well as in bound state. There is a great deal of controversy regarding of ? - amino, n– butyric acid in bound state. Steward FC, et al. [13] held that it was a decarboxylation product of L-glutamic acid and was not a constituent of proteins. On the contrary, Venkataram CS, et al. [2] Natarajan S, et al. [14] as well as Crossan DF, et al. [3] reported it as a constituents of proteins.

Reddy SM, et al. [5] reported that L–threonine was absent in four species of Helminthosporium in free state but was detected in bound state. Agnihotri VP, et al. [7] reported the presence of L – threonine in the soluble fraction of some species of Aspergillus, while Venkataram CS, et al. [2] observed its presence in both soluble and insoluble form in the species of Fusarium. Bilgrami KS, et al. [4] reported the presence of D – L valine in bound state in two species of Phyllosticta only while it was lacking in all the species in free state. Shreemali JL, et al. [15] could not detect them in the species of Phyllosticta.

McAnelly CW, et al. [16] while working with several strains of Fusarium solani, reported that the cells of successful pathogens contained relatively less amount of detectable free

amino acids. He concluded that L – aspartic acid, L – glutamic acid, glycine and DL – alanine were in greater amounts in the weaker pathogens. Bilgrami KS, et al. [4] working with eight species of Phyllosticta had also made similar observations. Presence of D-L leucine, L – tyrosine, D-L serine and glutamic acid in bound state has been reported by in Tilletia caries.

Agnihotri VP, et al. [2] Chandra S, et al. [17] Shreemali JL, et al. [15] Chahal et al. [18] Kannaiyan JP, et al. [19] and Mehta P, et al. [20] have given an account of amino acids synthesized by fungi during their metabolism. Iyer RS, et al. [21] shows significance of amino acid profile in the chemotaxonomic studies of karatinophilic fungi.

Carbohydrates

Several mycologists including Broyles JW, et al. [22] Crossan DF, et al. [3], Tandon RN, et al. [23] Binod BL, et al. [24], and Parmar SMS, et al. [25] have worked as closely related fungi and their strains to know their hyphal contents. Choudhary DP, et al. [26] and Parmar SMS, et al. [25] reported that the three keratinophilic fungi viz, Gymnoascus reesii. Baron., Microsporum gypseum (Boed) Guiart and Grigorakis and Trichophyton indicum Randhwe and Sandhu differed in their amino acid and sugar contents.

Study of sugar analysis of fungal hyphae was performed in India by most of the eminent mycologists including, Tandon RN, et al. [27] Ghose AK, et al. [28], Kapoor IJ, et al. [29], Mohanraj DP, et al [30], Bisen PS, et al. [31] Raghunathan R, et al. [32] Laxminarayan P, et al. [33] and Chary JS, et al. [34] They have observed that sugar contents of fungal hyphae of closely related species also varies according to their mode of nutrition.

Organic Acids

Synthesis of organic acids by fungi was first confirmed by the classical studies of Wehmeyer C, et al. [35] Since then a large number of reports appeared and acids of different types have been recorded as metabolic products of fungi. The presence of organic acids in the mycelium of fungi and their possible role have received considerable attention in recent past. Bateman DF, et al. [36] reported that Sclerotium rolfsii secreted large amount of oxalic acid in liquid culture media and in infected leaves during pathogenesis. Several workers including Singh BP, et al. [37] Roy MK, et al. [38] Agarwal DK, et al. [39] and Thompson DP, et al. [40] have carried out chromatographic analysis of fungal hyphae for the detection of organic acids.

The accumulation of citric acid has been regarded by Lewis KF, et al. [41] and Cochrane VM, et al. [42] as an unsual modification of TCA cycle. Ehrlich F, et al. [43] demonstrated synthesis of fumaric acid by Rhizopus nigricans. Malic acid which is now known to be produced by a number of parasitic and saprophytic fungi, was first recorded by Wehmeyer c, et al. [44] in Aspergillus fumaricus. Later Raistrick H, et al. [45] recorded malic acid synthesis in several fungi.

Study of organic acid analysis of fungal hyphae of various fungi were carried out by some of the Indian mycologists including Tandon RN, et al. [46] Kapoor IJ, et al. [29] Mehta P, et al, [20], Prasad SS, et al. [47] Chary JS, et al. [34] Vyawahare Sv, et al. [12], Pachkhede AU, et al. [48] etc.

Materials and Methods

In this analytical study, different isolates of Aplosporella Speg. used were obtained from monosporic cultures. These are A. labiatae, A. rubiae, A. coniferae, A. citrae and A. brossimumii. These are designated as L, R, Co, Ci and B respectively. It was decided to analyse almost all amino acids, as this is an important participants of protein, only a few organic acids and carbohydrates were analysed for. The pilot experiments were done with paper chromatography. However, the main results are derived from column chromatography. Strong cationic resin (Dowex – 50) as well as strong anionic resin (Amberlite IR A 400) were used. The column was packed with material and ion exchange resin. The slurry of dried fungal material was carefully poured down a glassrod to avoid bubbles trapping if any. The suspension was allowed to settle and excess of solvent ran off. The process was repeated until the column had the requested height. The material was removed from the column by eluting with an appropriate solvent. The effluent from the column was collected into a series of tests tubes. Each fraction was then analysed.

System

For Protein : BAW = n – BuOH – HoAc – H_2O

(250 : 60 : 250 V/V/V)

, 1 – Butenol : Acetic acid : Water

For sugars : n – Butenol : Acetic acid : Water (4 : 1 : 5 V/V/V)

For organic acids : n – Butenol – formic acid – water 10 – 2 – 15.

The contents of amino acids, sugars and organic acids in five isolates of Aplosporella are given in (Tables 1-3) respectively.

S.N.	Amino Acids	L	R	Co	Ci	B
1	D – L alanine	+	+	-	-	+
2	L – arginine	+	-	+	-	+
3	D – L aspartic acid	+	-	+	+	+
4	L – cystein	-	-	+	+	-
5	L – cystine	-	-	-	+	-
6	L – glutamic acid	+	+	-	-	-
7	Glycine	-	+	-	-	+
8	L – histidine	+	+	+	-	-
9	L – hydroxyproline	-	-	-	+	-
10	L – leucine	-	+	-	-	+
11	L – lysine	-	-	+	+	-
12	D – L isoleucine	-	+	-	-	+
13	D – L methionine	+	+	+	+	+
14	L – ornithine	-	+	-	-	-
15	D – L phenylalanine	+	+	-	-	+
16	L – proline	+	+	-	-	-
17	D – L serine	-	+	-	-	+
18	D – L threonine	-	-	+	-	-
19	D – L tryptophan	+	+	+	+	+
20	L – tyrosine	-	+	-	+	-
21	D – L valine	-	+	-	-	+

+ = Present - = Absent

Table1: Amino acid contents in five species of *Aplosporella*.

S.N.	Sugars	L	R	Co	Ci	B
1	Glucose	+	+	+	+	+
2	Fructose	+	-	+	+	-
3	Sucrose	-	-	+	-	+
4	Maltose	+	+	-	-	+

+ = Present - = Absent

Table 2: Sugar contents in five species of *Aplosporella*.

S.N.	Organic acids	L	R	Co	Ci	B
1	Fumaric acid	+	+	-	-	+
2	Malic acid	-	+	-	-	+
3	Malonic acid	+	-	-	+	-
4	Citric acid	+	-	+	-	-

+ = Present - = Absent

Table 3: Organic acid contents in five species of Aplosporella.

Results and Discussion

Amino Acids

It is evident from Table 1 that D – L methionine and D – L tryptophan are invariably present in all the five species of Aplosporella. D – L alanine, L – glutamic acid, D – L phenylalanine are identified in three species viz., Aplosporella labiatae (L), A. rubiae and A. brossimumii (B). L – cystine and L – hydroxyl – proline are detected in only one species viz A. citrae (Ci) while A. coniferae (Co) shows presence of D – L threonine. A. labiatae (L) and A. rubiae (R) revealed presence of L – proline and L – ornithine are detected in species viz A. rubiae (R) and A. coniferae (Co) and A. citrae (Ci) has L – cystein and L – lysine. Whereas L – tyrosine is present only in A. rubiae (R) and A. citrae (Ci). Glycine, L – leucine, D – L isoleucine, D – L serine and D – L valine are detected in A. rubiae (R) as well as in A. brossimumii (B).

Carbohydrates

In the present investigation an attempt was made to study the sugar composition of the hyphae of five species of Aplosporella. It appears from Table 2 that all four sugars were detected in the cultures of the five species of Aplosporella. Glucose was detected in all the five species. Fructose was detected in A. labiatae (L), A. coniferae (Co) and A. citrae (Ci). Sucrose was present in A. coniferae and A. brossimumii (B). Maltose was detected in three species viz. A. labiatae (L), A. rubiae (R) and A. brossimumii (B).

Organic Acids

Fumaric acid was detected in A. labiatae (L), A. rubiae (R) and A. brossimumii (B). Malic acid was found in A. rubiae (R) and A. brossimumii (B). Malonic acid was found in A. labiatae (L) and A. citrae (Ci) while citric acid appears in A. labiatae and A. coniferae (Co). It was observed that no species revealed all the four organic acids. Vywahare (1988) in his investigations of chromatographic analysis reported that these four organic acids were detected in Drechslera rostrata.

Conclusion

The isolates L, R and B are probably closely related as is indicated by the presence of some common metabolites. Among the above said three, isolate R and B have more affinity than L as about 14 metabolites are similar. Relationship between Co and Ci probably is close as seven metabolites are common.

The author strongly feels such studies will be of immense help in speciation of fungal forms. Host relationship is an

important aspect of classification and speciation in fungal identification. Such biochemical studies may open new vista in the criterion of host relationship in fungal speciation.

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