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BIOLOGICALLY ACTIVE PEPTIDES FROM *ASPERGILLUS FLAVIPES*

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Aspergillus flavipes was grown on Czapek sucrose nitrate medium for two weeks. The peptides from cell free broth (CFB) were separated on Sephadex G-25 column. Some of the peptides showed antimicrobial activities inhibiting the growth of fungi and bacteria. The amino acid composition, N-terminal amino acids and isoelectric points of these peptides are also reported.

Key words: Antimicrobial Activity, peptides, *Aspergillus.*

INTRODUCTION

The genus *Aspergillus* consisting of over 150 species (1) is well known for producing a number of metabolites of biological importance (2). Enzymes are the most extensively studied amongst these metabolites (3). However, the role of peptides synthesized by the species of *Aspergillus* has not been studied in detail.

A. flavipes grows in soils of tropical and humid areas. The fungus produces colonies which are white or silvery white, but changes to pale yellow or to greyish buff on ageing. The conidia are colourless, smooth, globose and 2-3 mm in diameter. A review of literature reveals that so far only few toxins [4-6], enzymes [7, 8] alcohol and ester [9] and a pigment [10] have been isolated and studied. The culture of *A. flavipes* produces a complex of multiple enzyme system which oxidises a variety of organic substances such as steroids [11], cyclopentaphenanthrene [12], glaucine [13] and imipramine [14]. An isolate from soil is reported to show inhibitory properties against other soil fungi [15]. Studies were therefore carried out to examine the antimicrobial activities of *A. flavipes.* Peptides (have been isolated from cell free broth of the culture of *A. flavipes.* The chemical analysis of the peptides like amino acid composition, electrophoresis, isoelectric focussing, and N-terminal assay of these peptides are presented.

EXPERIMENTAL AND RESULTS

A. flavipes (K.U.M.H. Culture No. 240) was used in these studies. Chemicals of analytical grade from E. Merck were used and solvents were distilled wherever necessary. *A. flavipes* was grown on Czapek sucrose nitrate medium [16] in 250 ml conical flasks at 37°. After 2 weeks growth of the fungal-mass and broth were separated by filtration centrifuged (5000 rpm). The clear dark brown supernatant was freeze dried and the fine brown powder obtained was kept in a deep freezer for further analysis.

Dried brown powder (500 mg) was dissolved in 50 ml of distilled water and separately extracted with benzene, ethyl acetate, and chloroform. The organic solvent extracts were discarded. An equal volume of ethanol was added to the aqueous layer to precipitate the high molecular weight compounds. The precipitated matter was separated by centrifugation (4000 rpm). The coloured supernatant was concentrated to 5 ml on a rotary evaporator at 50°. The proteins precipitated by ethanol settled at the bottom after centrifugation at 4000 rpm.

Gel permeation chromatography. The concentrated syrup was subjected to Sephadex G-25 column (170 x 2.6 cm) using 0.05 M CH_3COOH as eluent at a flow rate of 50 ml/hr. The void volume was found to be 418 ml. The elution pattern of the peptide is shown in Fig. 1.

Electrophoresis. Electrophoresis of the peptides was carried out in 10 % gel with 0.29 % cross linking using electrode buffers as Tris-gly pH-8.8 and Tris-HCl pH-8 with a current of 5mA/tube for 2 hours which produced a single band.

Isoelectric focusing. Isoelectric points of some peptides were determined by the isoelectric focusing method of Wrigley [17] using ampholine as carrier ampholites (Table 1).

N-terminal moiety. N-terminal moiety of amino acids of some peptides (Table 1) were determined by dansylation method of Gray and Hartlay (18).

Amino acid composition. Amino acid analysis was carried out by hydrolysing 1 mg of each peptide in a

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sealed tube in 5.7 HCl at 110° for 24 hr. HCl was removed in vacuum and the sample was redissolved in 1 ml of 0.2 M sodium citrate buffer pH 2.7. It was filtered and amino acid contents were determined by using Biotronik Automatic Amino Acid Analyser. LC 5001 (Biotronik Munich, West Germany).

The results are compiled in Table 1.

Biological activity. The microbial activities of the peptides were determined and some of the peptides were found to exhibit strong antifungal and antibacterial activities.

(a) *Antifungal activities:* Two mg each of the samples A-E were dissolved in 1 ml double distilled deionized sterilized water and 0.2 ml of each of the solutions were poured in a ceramic well placed on Czapek sucrose nitrate agar in Petri dishes. The dishes were inoculated with test fungi viz., *Fusarium solani, Helminthosporium sativum, Alternaria solani, Aspergillus flavus* and *Aspergillus niger* and incubated at 25°. Zones of inhibition observed are given in Table 2. Fig. 2 shows the zone of inhibition produced by peptide E.

(b) *Antibacterial activities:* The seed-plate method was used for testing the antibacterial activity, using (a) nutrient broth, (b) base layer and (c) seed layer media. The peptides were tested against *Staphylococcus aureus, Salmonella typhi, Shigella dysenteriae, Pseudomonas aeroginosa* and *Streptococcus lactis.*

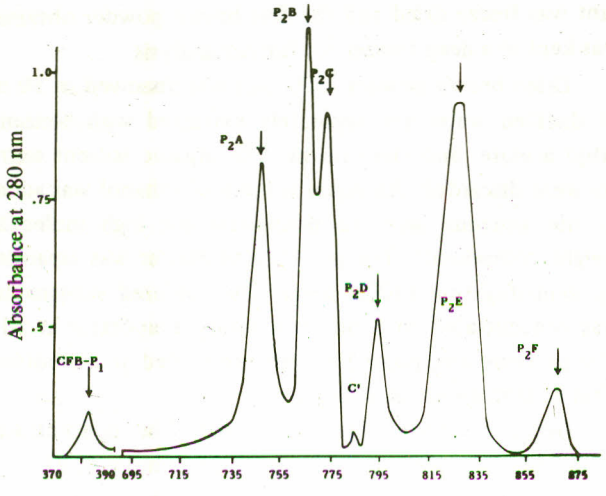

Fig. 1. Elution profile of the separation of CFP-P_2 fraction of *Aspergillus flavipes* on Sephadex G-25 Column (170 x 2.6) cm. eluted with 01M CH_3COOH at a flow rate of 50 ml/hr.

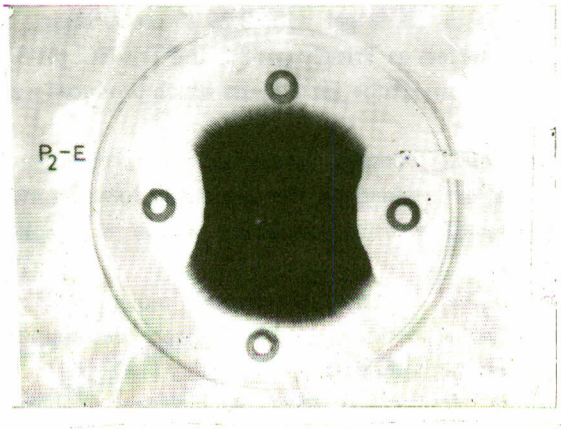

Fig. 2 Antifungal activity of peptide E.

Table 1. Amino acid composition (residue/1000) PI and N-terminal amino acids of peptides

Amino acids	P–2A	P–B	P–C	P–D	P–E
Asp	493.17	207.29	204.14	235.91	234.52
Thr	0.48	38.40	25.87	36.56	24.07
Ser	32.10	32.27	39.19	30.64	59.53
Glu	49.48	52.79	50.64	47.75	64.17
Pro	30.88	103.72	71.60	30.55	30.20
Gly	94.65	96.72	255.22	122.79	182.66
Ala	74.25	63.32	51.04	51.92	97.31
Cys	8.13	17.69	1.21	–	39.63
Val	33.89	48.78	77.36	62.03	32.03
Met	15.17	119.99	32.17	108.84	20.17
Ile	11.29	38.08	50.29	42.70	20.21
Leu	22.83	30.70	44.74	47.67	30.74
Tyr	8.18	27.03	26.74	27.56	37.43
Phe	7.90	52.01	19.54	39.43	28.34
His	98.48	39.10	49.81	87.02	73.65
Lys	19.09	32.08	0.36	27.54	23.99
Arg	–	–	–	–	–
PI	6.4	–	5.1	–	5.5
N-terminal	Asp	–	–	Asp	–

Table 2. Antifungal activity of the peptides isolated from *Aspergillus flavipes*

Test peptides	Test Organisms			
	Fusarium solani	*Helmintho-sporium sativum*	*Alternaria solani*	*Aspergillus flavus*
	(Zone of inhibition in mm)			
A	6	12	5	3
B	0	0	0	0
C	0	0	0	0
D	3	13	0	7
E	3	8	6	4

Data is average of 3 replicates

Table 3. Antibacterial activity of the peptides isolated from the CFB of *Aspergillus flavipes*

Test peptides	Test Organisms				
	Staphylococcus aureus	*Salmonella typhi*	*Shigella dysenteriae*	*Pseudomonas aeroginosa*	*Streptococcus lactis*
	Zone of inhibition in mm				
A	10	13	0	0	0
B	0	10	10	0	0
C	10	11	10	0	0
D	0	0	0	0	0
E	8	11	10	0	0

The nutrient broth was inoculated with the test organism and incubated at 37° for 24 hr. The cultures were then standardized by turbidometric method to obtain a standard amount of inoculum. Melted base layer medium (20 ml) was evenly distributed in each petri dish and allowed to solidify. The standardized inoculum was added to the melted seed layer medium at 40° and thoroughly mixed. The inoculated seed layer medium was then thoroughly mixed. The inoculated seed layer medium was then spread evenly on basal layer medium in each Petri dish. The plates were punched in the centre with the help of sterilized standard cork borer and 0.1 ml of peptide solution of known dilution was added to each hole and incubated for 48 hours. The zones of inhibition in mm are reported in Table 3.

DISCUSSION

In the present study, 5 new peptides have been isolated from the cell free broth cultures of *A. flavipes*. Some of these peptides when tested against certain fungi and bacteria produced a prominent zone of inhibition. Of these, peptide P_2-A was active against the fungi viz., *Fusarium solani, Helminthosporium sativum Alternaria solani* and *Aspergillus flavus*. Peptide P_2D exhibited high toxicity against *H. sativum* while was mildly toxic to *F. solani* and *A. flavus* and nontoxic to *A. solani*. Peptide P_2E also inhibited the growth of all the four fungi. It was interesting to note that peptide P_2B and P_2C did not exhibit any antifungal activity, but produced prominent zones of inhibition against *Salmonella typhi, Shigella dysenteriae*. P_2C was also active against *Staphylococcus aureus*. Similarly P_2D was nontoxic to all the 5 bacterial cultures tested, but produced zone of inhibition against *F. solani, H. sativum* and *A. flavus*.

The biological activities of the peptides revealed that certain peptides such as P_2A, P_2D and P_2E may play a vital role in food preservation against deadly toxic organisms like *A. flavus* and other, commonly present fungi and bacteria, responsible for food deterioration. Another important aspect in the use of these peptides would appear to be in the manufacture of certain fungicidal substances for the control of plant diseases caused by *H. sativum, F. solani* and *A. solani*.

Data on the amino acid composition shows that arginine is completely missing from all the peptides while cystine is not present in P_2D. All other peptides are also low in cystine contents. Aspartic acid is unusually high in all peptide especially in P_2A. The five peptides would appear to have great biological significance.

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