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ANTIBACTERIAL ACTIVITY OF PEPTIDES ISOLATED FROM *CANDIDA TROPICALIS*

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Antibacterial activity against gram positive and gram negative bacteria of 9 peptides isolated from *Candida tropicalis* cell free broth is reported. Four peptides of distinct amino acid composition were found to be active against a number of pathogonic and non-pathogenic bacteria.

INTRODUCTION

Numerous studies have been carried out on the fungus *Candida tropicalis*, belonging to the family Crytococcaeae, and known for producing candidiasis in human and animals. Studies on its chemical composition have revealed a number of components, such as vitamins [1], free amino acids [2], enzymes and co-enzymes [3], lipids [4] and antigens [5]. Various facets of its biological activity have been explored for industrial and medicinal purposes [6, 7].

Work on proteins has shown the presence of a glyco-protein, a nucleo-protein, a non-haem iron protein [8], and a flavo-protein. Alimova *et al.* [9] have isolated polysaccharides and lipo-polysaccharide; the latter exhibited some pyrogenic activity, attributable to its lipid fraction. Milehenko [10] and Kovalenko *et al.* [11] have detected in aqueous extract of *Candida tropicalis* an anti-viral component active against the tobacco mosaic virus and the potato-X-virus respectively. The later was established to be a poly-saccharide containing glucan and mannan in ratio of 1:4. There have been no reports so far on the anti-bacterial activity of *C. tropicalis.*

In this communication, we reporting for the first time the isolation of peptides possessing anti-bacterial activity against gram positive and gram negative bacteria.

EXPERIMENTAL AND RESULTS

Candida tropicalis culture was obtained from Central Bureau Voor Schimmel Cultures, Yeast Division, Barn, Holland. All chemicals were of E Merck Analytical grade and were distilled wherever necessary. The Sabouraud's dextrose broth (Oxoid) were used.

Cultures of pathogenic and nonpathogenic bacteria were obtained from Abbasi Shaheed Hospital, Karachi and the Department of Microbiology, University of Karachi, Karachi. The bacteria used in this study are shown in Table 1 A and 1B.

Candida tropicalis was grown in Sabouraud's dextrose broth [12] in a shaking water bath maintained at 37° for 7 days. After maximum growth of the fungus, cells were separated by centrifugation (4000 rpm) and washed 3-4 times with sterile distilled water. The cell-free culture broth was set aside for further studies.

Precipitation of proteins/peptides. About 1 litre of cell-free culture broth was concentrated *in vacuo* to 100 ml. protein/peptides were precipitated by the addition of ethanol. The supernatant was drawn off, concentrated and kept separately.

Separation on Sephade: G-100. The ethanol-precipitated fraction containing peptide/protein free amino acid, polysaccharide etc. was subjected to a column of Sephadex G-100 (2.5 x 60 cm) and eluted with 0.2 M acetic acid. Fractions of 1 ml each were collected on LMB fraction collector Ultrorac 7000 at a flow rate of 8 ml/hr. UV-absorbence was recorded at 206 and 254 by LKB detector UV-Cord III (LKB, Graefelfing, West Germany). The elution pattern is shown in Fig. 1.

Two peaks CTE_pS_1 and CTE_pS_2 were observed within the first void volume. Studies relating to these will be reported elsewhere. Another set of eleven unresolved peaks (CTE_pS_3-S_{13}) appeared near the end of the second void volume. These were pooled together, labelled as CT-E_p-F and freeze-dried.

Separation of CTE_p-F on Dowex 50 x 2. The concentrated peptide fraction CTE_p-F was subjected to a column of Dowex 50 x 2 (45 x 2.0 cms) and eluted with pyridine acetate buffer (0.02 M pyridine) using a stepwise linear pH gradient of 2.8-3.2 as starting buffer followed by 3.2-4.0,

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Table 1A

Peptide/s mg/ml Baterial strain	Kana mycin 12.5 mg/ml	D_1 16.31	D_2 11.6	D_4 6.8	D_5 15.93	D_7 6.06	D_9 9.33
1. *Bacillus megatherium*	+++	+	+	–	–	+	–
2. *Bacillus subtilis*	+++	–	–	–	–	+	++
3. *Bacillus thurengensis*	+++	++	–	–	–	–	
4. *Corynebacterium hofmanii*	+++	++++	+	+	+	+	+
5. *Micrococcus lysodeixticus*	+++	++	–	–	–	–	–
6. *Sarcina lutea*	+++	++	+	–	–	–	++
7. *Staphylococcus aureus*	+++	+	+	–	–	+	–

\+ Zone of inhibition up

\+ Zone of inhibition upto 10 mm ++Zone of inhibition upto 15 mm +++Zone of inhibition upto 25 mm

++++ Zone of inhibition upto 35 mm Peptides D_3, D_6 and D_8 did not show any inhibitory action

– No zone of inhibition

Table 1B

Peptide/s mg/ml Baterial strain	Kanamycin 12.5 mg/ml	D_1 16.31	D_2 11.6	D_4 6.8	D_5 15.93	D_7 0.06	D_9 5 9.33
1. *Enterobactor aerogenes*	+++	+	–	–	–	+	+
2. *E. coli (KL 16)*	+++	+	–	–	+	+	–
3. *E. coli (communis)*	+++	+	–	–	+	+	–
4. *E. coli (communoir)*	+++	–	–	–	+	+	++
5. *E. coli (8848)*	+++	–	+	–	–	+	–
6. *Klebsiella pneumonae*	+++	+	++	–	+	+	–
7. *Neisseria catarrhalis*	+++	++	++	+	+	+	++
8. *Proteus mirablis*	+++	+	+	–	–	–	+
9. *Proteus morganii*	+++	–	+	–	+	+	+
10. *Proteus vulgaris*	+++	+	++	–	–	–	–
11. *Pseudomonas aeroginosa*	+++	+	+	++	++	++	+
12. *Salmonella typhi*	+++	++	++	+	–	+	++
13. *Salmonella typhi A*	++++	++	+	+	–	+	+
14. *Salmonella typhi B*	+++	+	–	–	+	+	–
15. *Salmonella typhi* LT_2	+++	++	–	–	+	+	–
16. *Shigella sonnei*	+++	–	+	–	+	+	.
17. *Salmonella typhi 1978*	+++	+	–	–	–	+	–

\+ Zone of inhibition upto 10 mm

++ Zone of inhibition upto 15 mm

+++ Zone of inhibition upto 25 mm

++++ Zone of inhibition upto 35 mm

– No zone of inhibition

Peptides D_3, D_6 and D_8 did not show any inhibitory action.

4.0-5.0 and finally 5.0-6.8; the temperature was maintained at 55°. Fractions of 2.5 ml each were collected at a flow rate of 30 ml/hr. An aliquot were drawn off from alternate fractions hydrolysed in 6N HCl and treated with ninhydrin. The optical density was determined at 570 nm using Spectronic-20. Fig. 2 shows the elution pattern. Ninhydrin-positive fractions were pooled and concentrated *in vacuo*, taken in distilled H_2O and labelled as shown in the Fig. 2.

Antibacterial Activity. The cultures of bacteria grown overnight at 37° were used for testing the antibacterial activity of different fractions separated on sephadex G-100 and Dowex 50 columns. The assay was carried out by agar overlay and seed-plate method. The following are the details:

1. Overlay agar preparation: The plates were formed with two different percentages of agar. The thick hard layer or underlay was made with "Oxoid" nutrient medium containing sterilized 1.5% agar. The soft-agar overlay medium was prepared with 0.6% agar (pH 7). Aliquots of 2.5 m were sterilized and stored in refrigerator till required. As needed the soft agar tubes were melted and kept in a water bath at 45°C. The culture was added to soft-agar, mixed well, poured on sterile hard agar plantes and left for an hour at room temperature. Drop, disc and well methods were employed for testing the *C. tropicalis* fractions. Kanamycin (12.5 mg/ml) was used as a positive standard results are reported as zones of inhibition after 24-36 hours of growth at 37°C.

2. Seed-plate method: In this technique [15] Oxoid nutrient medium containing 1.4% agar was adjusted to pH 7, distributed in 40 ml quantity in screwcapped bottles and sterilized. The bacterial culture was then added aseptically to the agar medium at 45°C, mixed well and poured immediately in sterilized petri-plates. After hardening, wells were cut into agar and the *C. tropicalis* fractions were placed in these wells. The plates were incubated at 37°C, and observations made after 48 hours.

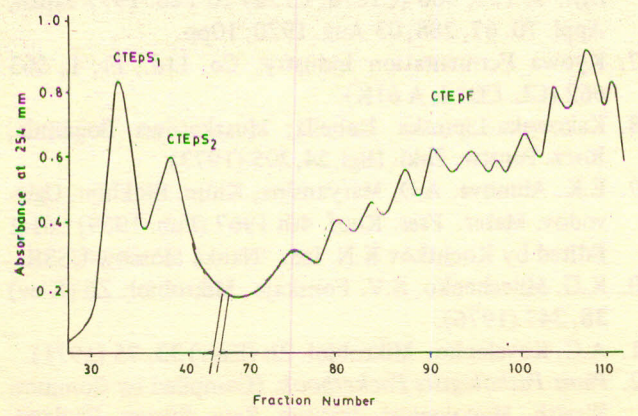

Fig. 1. Separation profile of ethanol precipitato fraction of cell free broth on Sephadex G-100.

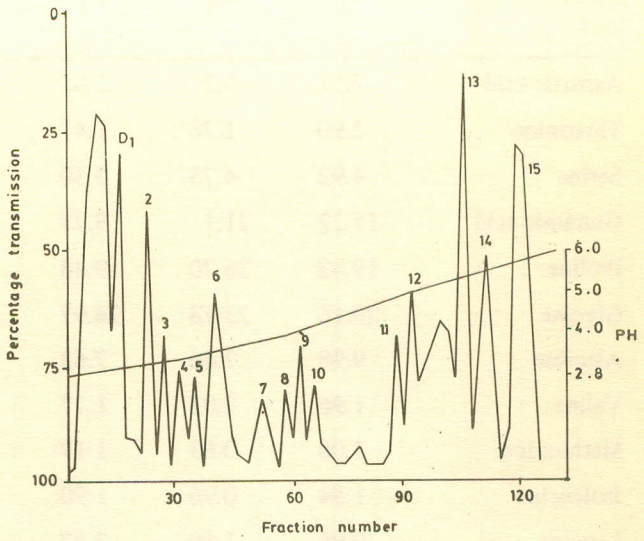

Fig. 2. Elution pattern of CTE_pF on Dowex 50 x 2.

Amino Acid Composition. 1mg of each peptide fraction from Dowex-50 was hydrolysed with 6N HCl at 110°C un-vacuum in a sealed tube at 110°C. The hydrolysate was vacuum dried, taken in sodium citrate buffer pH 2.2, and subjected to analysis on Biotronik Amino Acid Analyser LC 6001 (Biotronik GmbH, Munich, West Germany). The results are shown in Table 2.

DISCUSSION

Studies on cell-free culture broth of *C. tropicalis* have resulted in the isolation of 4 different peptide fractions which are active against a number of pathogenic and non-pathogenic bacteria. Clear zones of inhibition were observed after incubation at 37°C for 24 to 48 hours. Dl and D9 were active against 19 strains out of 24 tested where as D2, D4, D5 and D7 were active against 14, 3 and 10 strains respectively. D1 and D9 are interesting peptides because of their activity against a broad spectrum of bacteria. Furthermore, all peptides have been found to be inhibitory for *Neiserria catarrhalis* and *Cornyvebacterium haffamanni.*

All the active peptides have distinct amino acid compositions. The peptide 1 and 9 have glycine and alanine in similar quantities but possess a very high content of arginine. These two do not contain Tyr and Arg. The N-terminal amino acids were found to be valine and glutamic acid respectively. The N-terminal amino acids of the peptides are shown in table 2. D-7 appears to be a mixture

Table – 2

Amino acid composition of peptides (moles/100 moles)

Amino acid	D_1	D_2	D_3	D_4	D_5	D_6	D_7	D_9
Aspartic acid	7.53	6.3	5.05	5.19	4.50	4.4	5.16	6.70
Threonine	2.90	1.78	1.47	1.83	1.68	1.65	1.71	3.48
Serine	4.92	4.75	3.50	3.21	2.53	3.88	2.82	4.28
Glutamic acid	17.22	21.1	8.77	7.19	13.68	10.10	15.88	8.14
Proline	19.42	26.70	9.48	11.91	12.74	8.30	11.95	7.30
Glycine	29.16	23.28	24.97	37.86	33.3	21.67	27.6	31.94
Alamine	9.98	7.46	7.63	15.67	9.28	7.72	8.97	11.24
Valine	1.96	2.02	1.77	2.83	2.34	2.13	2.31	2.68
Methionine	1.08	0.83	1.19	0.42	0.49	2.66	0.61	1.63
Isoleucine	1.54	0.96	1.90	1.58	1.18	2.20	1.3	1.74
Leucine	0.96	1.69	2.57	2.28	2.66	2.73	2.57	3.20
Tyrosine	–	–	0.54	–	0.18	–	0.31	–
Phenylalanine	0.81	1.01	6.88	1.16	1.15	0.85	1.07	0.65
Histidine	1.06	0.6	2.69	0.8	1.11	2.29	1.13	1.79
Lysine	0.19	–	1.04	2.62	0.54	1.34	0.55	0.91
Arginine	1.18	1.39	20.48	5.28	12.53	28.01	15.9	14.24
N-terminal	Val, Ser Ser		Glu	Ser/Gly				

containing serine and glycine where as others have a single DNS-amino acids.

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