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ANTI-PHAGE Mu ACTIVITY AND MUTAGENICITY OF EXTRA CELLULAR PEPTIDES OF *CANDIDA TROPICALIS*

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Abstract

Genetic activity of peptides isolated from cell-free culture filterate of dimorphic yeast *Candida tropicalis* (strain CBS94) was investigated in Salmonella/microsome reversion assay. The peptides induced either base-pair substitutions or frame-shift mutagenesis and one of this promoted both kind of mutations. A peptide also inhibited Mu induced lysis of *Escherichia coli*, indicating that the peptide could interfere with the growth of a bacterial virus.

Introduction

Yeast-like fungi belonging to the genus *Candida* are known to cause Candidiasis in man and other animals (Dobos, 1991). Their cellular constituents as well as extra-cellular metabolites have been biochemically characterized and their biological activity examined (Negro, 1973; Sattarelli, 1981). Aqueous extracts of *Candida tropicalis* showed the presence of an anti-viral component active against tobacco mosaic virus (Milchenko & Afonskaja, 1976) and potato-X virus (Kovalenko, 1971). The activity was associated with a polysaccharide containing glucan and mannan in 1:4 ratio. Peptides from the cell-free culture filterate of *C. tropicalis* strain CBS94 have been isolated and their antibiotic activity has been reported (Abbasi, 1983, Abbasi *et al.*, 1985). In this communication the mutagenicity of the peptide as demonstrated by their ability to induce base-pair substitutions and frame-shift mutations in Salmonella/microsomes reversion assay, and antiviral activity of one of the peptides(D4) against bacteriophage Mu is being reported.

Materials and Methods

Culture of *Candida tropicalis* CBS94 was used. Peptides from cell free culture broth were isolated and characterized as described earlier (Abbasi, 1983, Abbasi *et al.*, 1985).

E. coli strain HM8305, a Mu*cts* lysogen, was used to prepare phage lysate and strain KL16 was used as indicator. LB broth was used for cultivation of bacteria as well as diluent for phage (Luria & Burrows, 1957). Phage titer was determined as described in Razzaki & Bukhari (1975), and Bukhari *et al.*, (1977).

Anti-Mu activity assay of peptides: A lawn of indicator bacteria was prepared by mixing 0.1 ml of log phase culture of *E. coli* KL16 with 2.5 ml of top agar and pour

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ing it over a basal layer of LB agar in a Petri plate. After the overlay agar had solidified, 0.1 ml of Mu lysate containing 10 plaque-forming phage particles was spotted in each of the four pre-marked sectors of the plate. The spots were allowed to dry at room temperature. Sterile filter paper discs were placed over each spot and 50 ul aliquots of various dilutions of the peptide being tested were pipetted onto the discs. The plates were incubated overnight at 37°C. Phage replication was indicated by the appearance of a clear zone around the discs. Conversely, the anti-phage activity of the peptide was indicated by bacterial growth in areas adjoining the discs.

Salmonella/microsome reversion assay: Oxoid and Difco culture media were used. IRC-191 was from Terochem Laboratories and all other chemicals were from Merck. Rats and their feed were gifts from the PCSIR Laboratories, Karachi. For the preparation of microsomal fraction from rat-liver, rats were induced with Aroclor 1254 according to Kier *et al.,* (1974). *Salmonella typhimurium* LT-2 derived strains TA97, TA98, TA100, TA102 and TA1535 were used according to standard procedures (Maron & Ames, 1983).

Spot test for mutagenesis: To 2 ml of molten top agar at 45°C, 0.1 ml of nutrient broth culture of the tester strain and 0.5 ml of S9 mix were added. The contents were mixed and poured on minimal agar glucose plates. After the overlay had solidified the peptide solution was spotted in 10 ul quantity. The plates were incubated at 37°C for 48 h. Mutagenesis was detected by the appearance of histidine revertants around the peptide spot. When the number of revertants exceeded the spontaneous reversion frequency by a factor of 2, the peptide was regarded as capable of inducing mutations in this system. ICR-191, sodium azide, MMNG and 2-aminofluorine were included in control experiments.

Plate incorporation assay: To a tube containing 2 ml of top agar supplemented with histidine and biotin, 0.1 ml of an overnight culture of the tester strain were added, 0.5 ml of S9 mix and the quantity of the peptide as indicated in the figure. The mixture was briefly vortexed and poured over minimal glucose agar plates. After the top agar had solidified the plates were incubated at 37°C for 48 h. Number of histidine revertants was determined by counting the number of colonies. Samples which were tested without rat liver enzymes contained 0.5 ml of S9 mix components from which S9 had been omitted.

Results and Discussion

Peptide D4 was found to inhibit lysis of KL16 bacteria by phage Mu (Fig. 1 A,B). Lysis inhibition was dose-dependent. Anti-Mu activity of this peptide was not significantly affected by a 10- or 100-fold dilution. However, at a 1000-fold dilution, Mu promoted lysis was not arrested. This suggests that anti-viral activity is being simultaneously diluted as the peptide preparation; hence it is associated with the peptide D4. None of the other peptides was found to be active against Mu. Mutagenicity of peptides was initially screened by a spot test. On the basis of the results shown in Table I, dose response curves were constructed. From the data shown in Fig. 2a-j, the peptides can be classified in four groups on the basis of their ability to promote mutations detectable in the Salmonella/microsome reversion test.

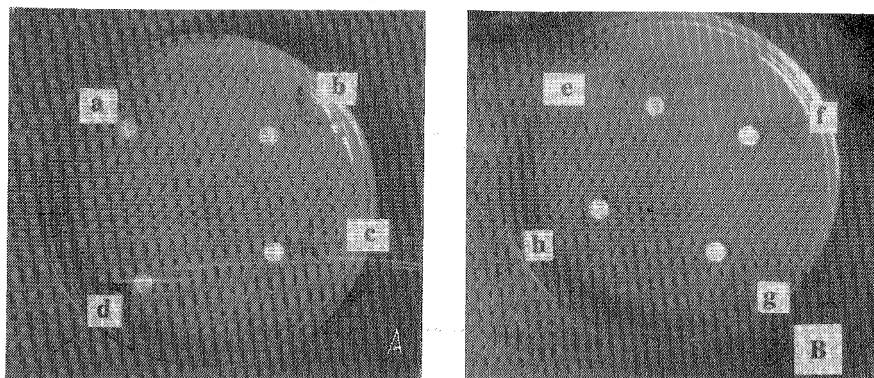


Fig.1. A & B. Antiviral activity of peptide D_4 showing arrest of Mu induced lysis of bacterium *Escherichia coli*:
a = Mu phage + D_4 (1:10), b = Mu phage + (1:0), c = Mu phage (control), d = Mu phage + D_4 (1:100), e = D_4 (control), f = Mu phage (control), g = Mu phage + D_4 (1:1000), h = Mu phage + D_4 (1:2000).

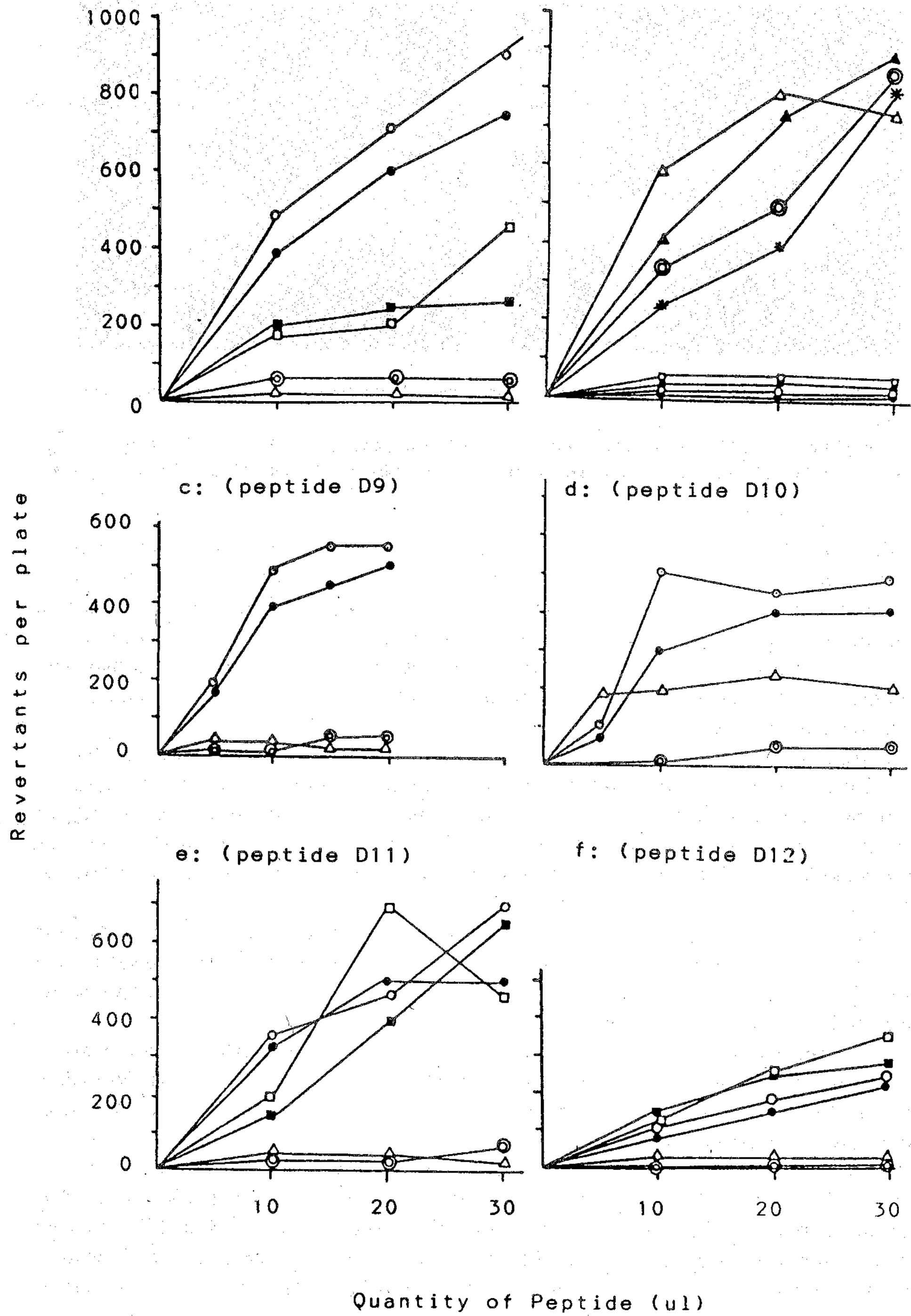
Peptides D1,D8-D12 and D15 induce frame-shift mutagenesis. Their genetic activity was detected by their ability to revert strain TA98 in the preliminary screening. Further confirmation was obtained with dose response studies. Where sufficient quantities of peptides were available additional information was gleaned by testing in strain TA97. Compared to other peptides, D12 is weakly mutagenic. Only two peptides D2 and D5 promoted base-substitution mutagenesis. Both the peptides were active in strain TA100 and TA102.

Peptide D13 promoted both frame-shift and base-substitution mutations. It is a powerful mutagen in TA97 and TA98 and a weak mutagen in TA100. Number of revertants generated through frameshift mutations in strain TA98 was 3.5 fold higher than the number of revertants induced through base-substitutions in TA100. The other peptides D3, D4, D6, D7, and D14 did not induce reversions in any of the five tester strains used in this study.

The mutagenic peptides do not appear to have a requirement for activation with rat liver enzymes. In the presence of S9, however, mutagenesis was enhanced from a slight to a moderate extent depending on the individual peptide and the tester strain in which the reversion is being monitored.

It is interesting to note that 10 out of 15 peptides have shown mutagenic activity. The amino-acid composition of the peptides reported by Abbasi *et al.,* (1985) does not offer any suggestions. The amino-acid sequences and their tertiary structures may provide the explanation for this behaviour.

Though the percentage of *C. tropicalis* infections in the overall incidence of Candidiasis is relatively low as compared to the number of infections due to *C. albicans*, nevertheless, it is disconcerting to note that majority of the peptides which we have examined are mutagenic. This finding adds a new dimension to the morbidity and mortality associated with *Candida* infections which are on the rise due to increasing use of anti-bacterial and cytotoxic drugs and immuno-suppressive therapy.


1000
800
600
400
200
0
c: (peptide D9)
600
400
200
0
d: (peptide D10)
Revertants per plate
e: (peptide D11)
600
400
200
0
f: (peptide D12)
10
20
30
10
20
30
Quantity of Peptide (ul)

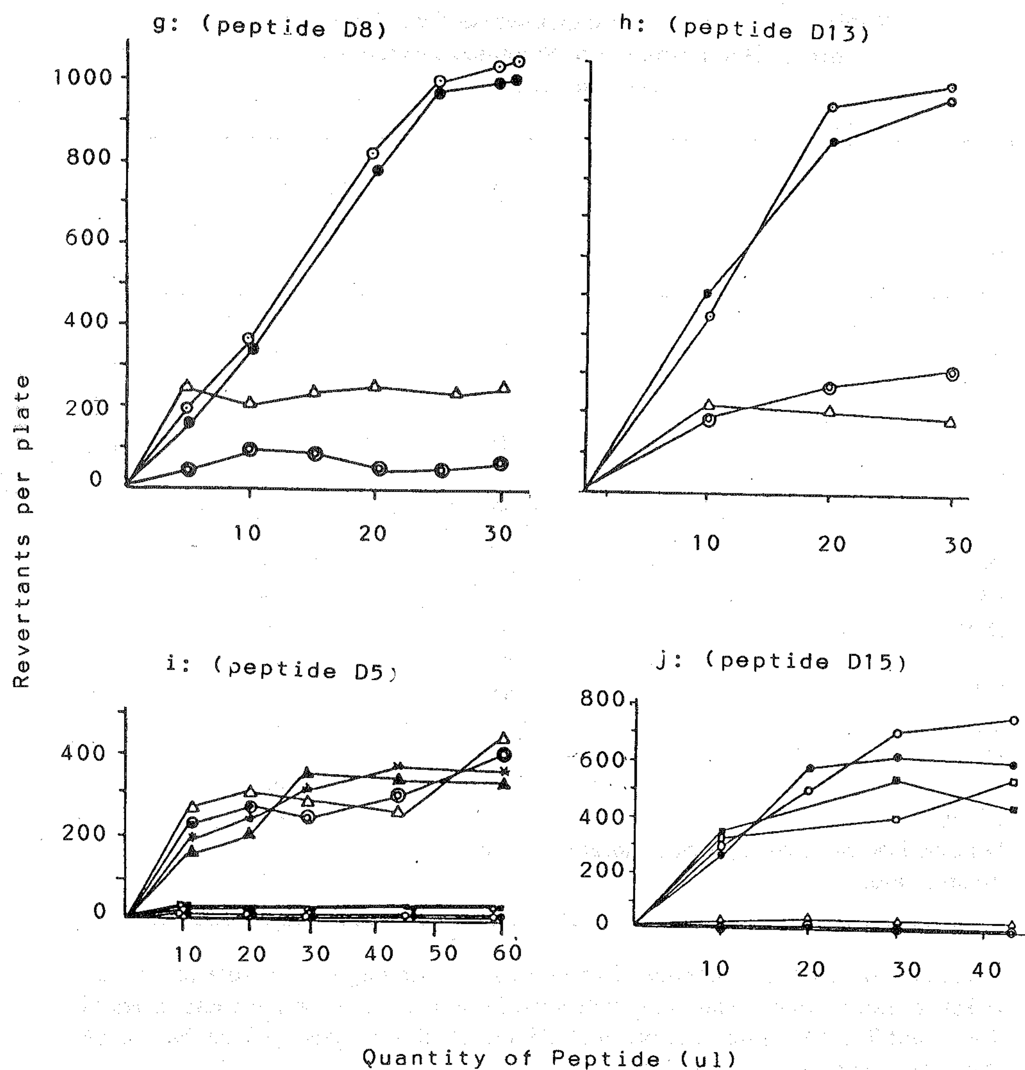


Fig.2. A-J: Mutagenicity of peptides in Salmonella/microsome reversion assay: The indicated quantities of peptides were evaluated for their ability to induce His- His+ mutation. The number of spontaneous revertants was substracted from the total number of revertants.

-●-●- TA98-S9, -○-○- TA98+S9; -■-■- TA97-S9, -□-□- TA97+S9, -✡-✡- TA100-S9, -◎-◎- TA100+S9, -△-△- TA102-S9, -▲-▲- TA102+S9

Table 1. Evaluation of mutagenicity of *Candida tropicalis* extracellular peptides in Salmonella/microsome reversion assay.

Peptide No.	REVERTANTS TA98	REVERTANTS TA1535
D1	α	-ve
D2	-ve	α
D3	-ve	-ve
D4	-ve	-ve
D5	-ve	45
D6	-ve	-ve
D7	-ve	-ve
D8	α	-ve
D9	α	-ve
D10	α	-ve
D11	α	-ve
D12	98	-ve
D13	α	60
D14	-ve	-ve
D15	α	-ve
ICR-191	50	80
N-methyl-N'-nitro-N-nitrosoguanidine	-ve	α
2-aminoflourine	α	50

α ,45,60 and 98 are the number of revertants surrounding a 10 μl spot of peptide being tested. α = more than 400 revertants. Spontaneous reversion frequencies of TA98 and TA1535 in our hands are 29-35 and 15-20 respectively. They have been subtracted from the values shown above.

ICR-191 and N-methyl-N'-nitro-N-nitrosoguanidine have been used as known mutagens for TA98 and TA1535, respectively. They were simply spotted as crystals.

All tests were done in presence of S9 rat liver microsomal activation system. 2-aminofluorine was used to monitor the efficacy of S9 preparation.

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