ISOLATION OF BIOCIDAL PEPTIDES FROM *Daucus carota* SEEDS

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*Two biocidal peptides were isolated from carrot seeds (*Daucus carota*). Their fungicidal activity toward the pathogenic fungus* Verticillium dahliae *was investigated.*

Key words: *Daucus carota* L. , biocidal peptides, wilt.

Peptides from the Umbelliferae family, to which carrot belongs, are practically unstudied.

The goal of the present work was to isolate biocidal peptides from carrot seeds (*Daucus carota* L., Apiaceae, Umbelliferae), establish their cationic nature, and determine their fungicidal activity.

The most effective extractant of peptides from ground and defatted carrot seeds was H_2SO_4 (0.05 N). The total protein extract was analyzed by gel electrophoresis in PAAG. Figure 1 shows that the crude total extract contained a large amount of low-molecular-weight peptides with molecular weights from 2.5 to 10 kDa. Next, the total extract was separated over a Sephadex G-15 column into fractions (Fig. 2). Each fraction was tested for fungicidal activity toward pathogenic strains of the fungus *Verticillium dahliae* Kleb. using a turbidimetric method [1]. The results from the determination of the fungicidal activity of the protein and peptide fractions from carrot seeds are given below:

Fraction	IC_{50} μg/mL
I	62.5
II	>100
III	-
IV	-
CS-1	18
CS-2	24.5

It was found that the first fraction had the highest fungicidal activity, IC_{50} = 62.5 μg/mL. According to the literature [2-4], cationic proteins have the highest fungicidal activity. Fraction I was separated by cation-exchange chromatography over a CM TSK-gel column equilibrated with ammonium—bicarbonate buffer (0.01 M, pH 6.2) into two components designated CS-1 and CS-2. The homogeneity of the isolated peptides was determined by capillary electrophoresis and gel electrophoresis in PAAG (Fig. 3). The molecular weights of CS-1 and CS-2 were 10 kDa. The determination of the fungicidal activity of CS-1 and CS-2 toward *V. dahliae* Kleb. showed that it more than tripled after ion-exchange chromatography.

According to the results, both isolated peptides CS-1 and CS-2 possess high fungicidal activity toward *V. dahliae* Kleb. Peptide CS-1 was purer than CS-2, which had a small impurity peak with retention time 8.18 min on capillary electrophoresis. These impurities and, possibly, the different nature of CS-2, produce the relatively lower fungicidal activity for CS-2 toward *V. dahliae* Kleb. wilt compared with CS-1. The biocidal activity of peptides isolated from carrot seeds is greater than that of analogous peptides from four Brassicaceae species (*Brassica napus*, *B. rapa*, *Sinapis alba*, and *Arabidopsis thaliana*) [5] and lower than that of horseradish (*Raphanus sativus* L.) [6]. Thus, two biocidal peptides with fungicidal activity were isolated from carrot seeds.

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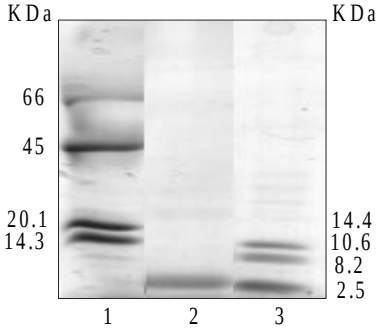

Fig. 1

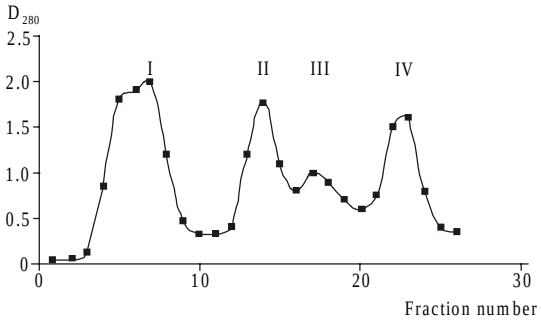

Fig. 2

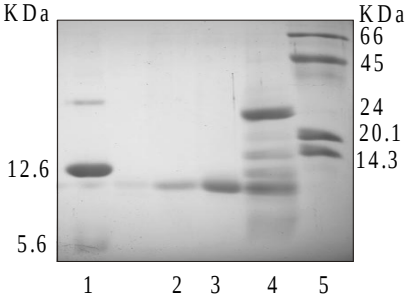

Fig. 3

Fig. 1. Gel electrophoresis of peptides from carrot seeds and standard proteins in PAAG (12.5%) of the isolated fractions: mixture of marker proteins (1), crude extract of carrot-seed peptides (2), mixture of marker peptides (3).

Fig. 2. Gel filtration of total protein extract of carrot seeds over a Sephadex G-15 column (2.5 × 85 cm) using ammonium-bicarbonate buffer (0.01 M, pH 6.2). Flow rate 60 mL/h.

Fig. 3. Gel electrophoresis of peptides and standard proteins in PAAG (12.5%): mixture of marker peptides (1), CS-1 peptide (2), CS-2 peptide (3), trypsin (24 kDa) (4), mixture of marker proteins (5).

EXPERIMENTAL

Isolation and Purification of Peptides from Ripe Carrot Seeds. Carrot seeds (50 g) were ground by the literature method [7] and defatted for 72 h with hexane in a Soxhlet apparatus. Proteins and peptides were extracted from the defatted seeds with H_2SO_4 (200 mL, 0.05 N) for 3 h at 30°C. The insoluble part of the seeds was removed by centrifugation at 6000 rpm for 15 min. The supernatant was neutralized with NaOH (10 N) and left at 5°C for 12 h until globulins had completely precipitated. Next the resulting precipitate was removed by centrifugation. The supernatant was dried by lyophilization to afford dry solid (6.52 g, 74.8%) containing peptides.

Separation of Total Peptide Fraction. The peptide fraction (400 mg) was placed on a Sephadex G-15 column (85 × 2.5 cm). The separation was performed using ammonium—bicarbonate buffer (NH_4HCO_3, 10 mM, pH 6.2). Fractions were collected, dried by lyophilization, and tested for fungicidal activity.

Ion-exchange Chromatography. Fraction I (150 mg) in starting buffer (140 mL) was placed on a TSK CM-650M column (1 × 5 cm) equilibrated with ammonium—bicarbonate buffer (10 mM, pH 6.2). The adsorbed peptides were eluted by a NaCl gradient (0-1.4 M). The combined peptide fractions were desalted over a Sephadex G-15 column equilibrated with NH_4HCO_3 buffer (10 mM), collected, and dried by lyophilization. The purity and molecular weight of the two isolated peptide fractions were determined by gel electrophoresis in PAAG.

Electrophoresis in PAAG (12.5%) was performed at pH 8.3 in the presence of sodium dodecylsulfate (0.1%). Gels were developed with coomassie R-250. The excess of dye was removed by washing with acetic acid (7%) in methanol (5%) [8].

Fungicidal activity of the isolated pure peptides was determined by a turbidimetric method [1] by measuring the decrease of optical density of suspensions of conidial spores of *V. dahliae* Kleb. at 550 nm in the presence of biocidal peptides compared with a control.

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