

## ***In vitro* Functional Analysis of Synthetic Cupep-1 and Cupep-2 Peptides from Phloem Sap of Chinese Long Cucumber**

**Hattem M. El-Shabrawi\*, Mohsen Asker<sup>1</sup>, Usama I. Aly, Hassan Ghareeb, Ahmed M. M. Gabr, Moemen S. Hanafy and Chun Ming Liu<sup>2</sup>**

*Plant Biotechnology Department, Genetic Engineering and Biotechnology, National Research Center, Dokki, 12622 Cairo, Egypt*

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### **Abstract**

The vascular system of plants consists fundamentally of two functional cell types, phloem and xylem. Phloem forms an extensive conduit for the dynamic long-distance transport of a diverse range of macromolecular signals that are required for plant development and defense mechanisms. Vascular tissues contain low levels of signaling peptide molecules, which are technically difficult to identify and characterize. We identified low molecular weight peptides from the phloem sap of Chinese Long cucumber using nano-LC-ESI-MS/MS with high SEQUEST scores. Bioinformatics analyses predicted their biological functions as signaling peptides and/or antimicrobial peptides. Here, we selected two peptides (Cupep-1 and Cupep-2), with highly SEQUEST score, to be synthesized and functionally investigated. Cupep-1 synthetic peptide showed a robust antimicrobial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis* and *Candida albicans*. These results potentiate the use of Cupep-1 peptide as a natural food preservative. We also show that the Cupep-2 peptide was able to conditionally change plant cell differentiation. Thus we suggest a role of Cupep-2 in plant development.

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\*Author for correspondence: <helshabrawi73@yahoo.com>. <sup>1</sup>Microbial Biotechnology Department, Genetic Engineering and Biotechnology, National Research Center, 12311 Cairo, Egypt. <sup>2</sup>Key Laboratory of Plant Molecular Physiology, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China.

## Introduction

In the vascular plants phloem is one of the primarily specialized tissues for translocation and distribution of organic metabolites produced during photosynthesis specially sieve elements which translocate wide range of organic metabolites (Stewart and Young 1984). In last decade researchers discovered the most important role of phloem and sieve elements in distribution of macro molecules like phytohormones, proteins and RNAs (Baker 2000, Jorgensen 2002). The expectance of macromolecules like protein and RNA in phloem was not expected because of the disability of sieve element to synthesize its own mRNA and proteins. Even though, the plant vascular scientist provides a strong evidence for the presenting of large number of RNAs and soluble protein in exudes substance of sieve element and companion cells which the protein synthesis are accrued (Lucas et al. 2001, Walz et al. 2004, Lough 2006). However, there are many phloem sap proteins or peptides have been discovered with specific roles in stress, defense and signal transduction (Kehr 2006). So, every living cell could have its own system to peptide synthesis, secretion, and posttranslational modification. These peptides are commonly used as signal molecules for intercellular communication in prokaryotes, fungi, animals and plant (Schaller 2001). Research in recent years, however, showed that peptides may also be widely used as chemical signals in plants. Moreover, plants do not only perceive endogenous peptide signals, but also react specifically to a large number of exogenous peptides produced by pathogenic microorganisms. Antimicrobial peptides (AMPs) are widely attracted as a candidates for plant protection research in the past decades (Montesinos 2007). More than 900 AMPs have been identified and classified according to their structures (1) linear peptides often adopting helical structures, (2) cysteine-rich open-ended peptides containing disulphide bridges, and (3) cyclopeptides forming a peptide ring. Linear and cyclic peptides may have linked fatty acid chains (lipopeptides) or other chemical substitutions, resulting in complex molecules (pseudo peptides) (Ramadan and Asker 2012). In nature, there are a large numbers of antimicrobial compounds that play an important role in the natural defense of all kinds of living organisms (Ramadan and Asker 2009). Food-borne illness caused by consumption of contaminated foods with pathogenic bacteria has been of great concern to public health. Controlling pathogenic microorganisms would reduce food-borne outbreaks and assure consumers a continuing safe, wholesome and nutritious food supply (Allahghadri et al. 2010). To determine antimicrobial activity of the Cupep-1, the peptide was tested against selected number of microorganisms that belong to different categories, Gram-negative and positive bacteria, yeast and fungi. This was assumed to be sufficient for the antimicrobial

screening. In this work, we investigated the *in vitro* biological activities of two different synthetic peptides, Cupep-1 and Cupep-2, selected from the extracted low molecular proteins of Chinese Long cucumber phloem sap and sequenced via LC/MS/MS using Chinese long cucumber data base. Then according to their predicted functions, we piked up those two peptide sequences to be synthesized based on their prediction roles in defense and development.

## Materials and Methods

The proteome sequence of (*Cucumis sativus*, Chinese Long) was provided by Dr. Sanwen Huang, Beijing Genome Institute (BGI)-Shenzhen, Chinese Academy of Science, Shenzhen, China.

The peptides Cupep-1 and Cupep-2 were chosen according to their predicted function as antimicrobial and developmental peptides. To those functions we were synthesized their protein sequences by GenWay Biotech, Inc. (San Diego CA, USA) with automated Solid Phase Peptide Synthesis (Tsuchiya et al. 1996). They were analyzed by reverse-phase high performance chromatography and MALDI-TOF mass spectrometry, which revealed higher than 85% purity.

The predictions of secondary structure (Garnier et al. 1987), hydrophobicity (Hopp and Woods 1981), antigenic index (Jameson and Wolf 1988), and surface probability were determined with the SEQUEST (Thermo Scientific, USA), ExPASy (<http://www.expasy.org/tools/>), and ProtParam servers (<http://web.expasy.org/protparam/>). All other predictions were made by using online services (PSORT (<http://psort.hgc.jp/>), signal IP 4.1 (<http://www.cbs.dtu.dk/services/SignalP/>) and MotifScan ([http://myhits.isb-sib.ch/cgi-bin/motif\\_scan](http://myhits.isb-sib.ch/cgi-bin/motif_scan)). Protein sequence alignments were performed with the Protein BLAST program (<http://blast.ncbi.nlm.nih.gov/>). The synthesized peptide Cupep-1 was diluted in deionized water to obtain a stock solution of 5 mg/ml as the final concentration for antimicrobial activity, while Cupep-2 was diluted in phosphate buffer (pH 7.0) to obtain a stock solution of 25nM/ml for plant growth activity.

*Antimicrobial activity:* Antimicrobial activity assays were carried out with conventional disk diffusion tests (Greenwood 1983) using cultures of *Bacillus subtilis* NRRL B-94, *Escherichia coli* NRRL B-3703, *Pseudomonas aeruginosa* NRRL, *Staphylococcus aureus* NRRL, *Aspergillus niger* NRRL313, *Saccharomyces cerevisiae* NRC and *Candida albicans* NRRL477. The bacterial strains were cultured in nutrient medium, while *Aspergillus spp.* and the two yeast strains were cultured on malt- and yeast medium, respectively. Broth media included the same contents except for agar. Bacteria and yeast were incubated for 24 hrs and *Aspergillus spp.* for approximately 48 h. The growth cultures of fungus were filtered by a thin layer of sterile sintered Glass G2 to remove mycelial fragments

before the solution containing the spores was used for inoculation. For preparation of inoculated plates, 0.5 ml of inoculant was added to 50 ml of agar media (50°C) and mixed by simple inversion. The media was poured into 12 cm dia Petri dishes and allowed to cool to room temperature. Sterile filter paper disks (2 mm in dia) were saturated with peptide solution (5, 10, 20, 30, 40, 50 and 60 µg/ml). Three saturated filter papers were fixed on the surface of the medium for each plate. The microbial growth inhibition zone was measured after incubation at 30°C for 24 hrs for yeast, 24-48 hrs for bacteria and 48-72 hrs for *A. niger*. The microbial inhibition zone was calculated as the mean of three replicates.

The culture medium (50 ml) was poured into Petri dishes (12 cm dia) and maintained at 45°C. The synthetic Cupep-1 peptide was added to the medium as 5, 10, 15, 20, 30, 40, 50 and 60 µg/25 ml, while constantly stirring to ensure a uniform distribution. From the suspension of different microbial strains, 30 µl were placed on the surface of the solidified culture medium containing Cupep-1. After the microorganism suspensions were absorbed into the medium, the plates were incubated at 30°C for 24-48 hrs. MIC was determined as the lowest concentration of Cupep-1 peptide inhibiting the visible growth of each organism on the agar plate. The microbial growth was monitored visually, and the MIC was determined according to Raaijmakers et al. (2006).

In order to investigate which molecules or processes Cupep-1 might target in order to be antimicrobially active, we tested 3.75 and 7.5 µg/100 ml, respectively, of the peptide on the biosynthesis of acid soluble phosphorus compounds, total lipids, proteins and nucleic acids (DNA and RNA) in the cells of *B. subtilis*. Immediately after inoculating flasks with *B. subtilis*, the peptide was applied in concentrations of 3.75 and 7.5 µg/100 ml media. Each experiment was repeated three times. Subsequently, the flasks were shaken (150 rpm) using a rotary shaker at 30°C. Samples were collected at 0, 20, 40, 60, 80, 100 and 120 min after incubation. The *B. subtilis* were subjected to the following tests: total acid soluble phosphorus, total lipids, total soluble proteins and total nucleic acids (RNA and DNA).

The bacterial cells were collected by centrifugation at 5000 rpm, washed twice with ice cold saline and subjected to two successive extractions with 5% ice cold trichloroacetic acid (TCA). The suspensions were centrifuged at 5000 rpm. One ml of the extract was added to 4 ml reagent (40 ml of 6 N H<sub>2</sub>SO<sub>4</sub>, 80 ml distilled water, 40 ml ammonium molybdate solution and 40 ml ascorbic acid), mixed and incubated at 37°C for 2 hrs and then cooled at room temperature. The absorbance (OD) was measured at 680 nm (Greenwood 1983).

The debris cells of *B. subtilis* were subjected to three successive extractions with a mixture of chloroform-methanol (2 : 1, v/v). A volume of 0.1 ml of extract was added to 5 ml of concentrated H<sub>2</sub>SO<sub>4</sub>. The mixture was heated for 10 min in a boiling water bath and cooled, and 0.4 ml aliquot was placed in a test tube. Six milliliters of phosphor-vanillin reagent (0.6 gm vanillin dissolved in 10 ml ethanol before diluting to 100 ml with distilled water and mixing with 400 ml of concentrated orthophosphoric acid) was then added to each test tube. The mixture was set in the dark for 45 min, and the absorbance (OD) was measured at 525 nm (Bligh and Dyer 1959, Knight et al. 1972).

The debris cells of *B. subtilis* were solubilized in 1 N KOH at 37°C for 20 hrs. The total protein in the extract was determined at 595 nm (Bradford 1976).

The remaining portions of the sample after hydrolysis by 1 N KOH were subjected to extraction of RNA fractions. To each sample, 6 N HCl was added, and then the solution was completed with the same volume of 10% TCA. After adjusting the concentration, the residue was washed with 5% TCA. One ml of RNA fraction was added to 3 ml of reagent (135 mg of ferric ammonium sulfate and 0.2 g orcinol dissolved in 15 ml distilled water, to which 85 ml of concentrated HCl were added), mixed and heated for 20 min in a boiling water bath. The tubes were cooled at room temperature and measured at 670 nm (Burton 1957, Montesinos 2007).

The residue after extraction of RNA was hydrolyzed by 5% TCA, and the supernatants were heated at 90°C for 30 min, cooled and centrifuged at 6,000 rpm. The residue was washed once with 5% TCA. One ml of DNA extract was added to 2.5 ml of the diphenylamine reagent (1 g of diphenylamine dissolved in 98 ml of glacial acetic acid to which 2 ml of H<sub>2</sub>SO<sub>4</sub> was then added), and the mixture was heated for 5 min in a boiling water bath. The sample was cooled, and absorbance was measured at 540 nm (Burton 1957, Montesinos 2007). All work was carried out under subdued light conditions. All experimental procedures were performed in triplets if the variation was less than 5% and the mean values ( $\pm$  standard deviation) were determined.

To test the impact of the peptide on plant development, two types of one month old *in vitro* globe artichoke (*Cynaracardunculus* var. *scolymus*) cultures (young shoots and undifferentiated callus) were used as the source for treatments. It is well known that the globe artichoke callus and shoots are usually viable for only short period. So, we want to test the effect of only Cupep-2 peptide on their viability. The callus was generated from globe artichoke explants according to (Brutti 2000, Bekheet 2011). One piece of callus (approximately 400 mg) was placed in 4.5 cm glass petri dish containing medium. The cultures were grown on MS medium (Raaijmakers et al. 2006)

supplemented with 3% sucrose (w/v) and 0.8% (w/v) agar. The filter-sterilized Cupep-2 peptide was added to the autoclaved medium at concentrations of 0, 25, 50 and 100 nM. The pH of all the media was adjusted to 5.8. The cultures were incubated at  $24 \pm 2^\circ\text{C}$  and relative humidity of  $50 \pm 5\%$  under a 16 hrs photoperiod regime with  $30 \mu\text{mol m}^{-2}\text{s}^{-1}$  photosynthetic photon flux density provided by cool-white fluorescent light tube. These conditions were the same for both testing plant types.

## Results and Discussion

Amino acid composition is a basic feature of protein sequence that defines a protein's secondary structure, folding type, subcellular location, and domain function. Here, we studied the potential functions of two peptides, Cupep-1 and 2 that were selected from the low molecular weight protein sequences of extracted proteins from the phloem sap of Chinese cucumber (*Cucumis sativus* L.) (Data not shown). It has been previously reported that most of the phloem sap proteins are involved in mediating developmental and defense responses such as redox reactions and proteases inhibition, (Kehr 2007, Kehr and Buhtz 2008). To predict a function of Cupep-1 and 2, we performed bioinformatics analyses of the two peptides. The isolated peptides Cupep-1 and 2 had a small molecular weight, 3.545, 3.507 KDa, respectively (Table 1). Both peptides have high SEQUEST ranking score, it may indicate that they have a trusted sequence, as long as the Delta CN Correlation value ( $\Delta\text{CN}$  score) near to  $\geq 0.2$  value (Table 1). The physico-chemical characterization analysis of Cupep-1 and Cupep-2 showed that the protein has 35 and 31 amino acids as unique peptides respectively (Table 2). It contained proline 23%, valine 17%, serine 14%, lysine 8.5% and 6% arginine (Fig. 1A).

The sequence homology search identified the Cupep-1 peptide as a defensin and saposin-like (Fig. 1A). This type of peptide can cross the cell membrane alone; therefore, it is called a cell penetrating peptide (Derossi et al. 1994). We found that Cupep-1 has a long half-life in mammalian, yeast and *E. coli* cells (100 hrs, > 20 hrs and > 10 hrs, respectively). The long half-life is potentially beneficial for use in the treatment of asthma, inflammatory diseases, MS, or pulmonary hypertension (Table 1). The sequence of Cupep-2 contained serine 20%, arginine 13%, glycine 9.5%, threonine 9.5 and 6.5% valine (Fig. 1-B). It has low GRAVY 0.165 (Table 1). The low GRAVY value indicates a good possibility for interaction with water. Cupep-2 shows 70% similarity to VIP2 protein via the sequence homology, which has a function in defensive cytotoxicity, pathogenicity, intracellular signaling, DNA repair and cell division (Han et al. 1999). This prediction would assign Cupep-2 multifunctional roles. The signature structure

analysis predicted Cupep-2 to be a mitochondrial targeting peptide. Thus it can be potentially conjugated with peptides that act against human mitochondrial diseases as a clinical application (Table 2).

**Table 1. The SEQUEST characterization data of Cupep-1 and 2.**

Peptide	MW (Da)	FDA ( $\geq$ )	XCorr	dCN	Trust status	Annotation
<b>CuPep-1</b>	3.55	0.1	3.570	0.213	Excellent	Aldo/keto reductase family oxidoreductase soluble NAD(P)(H) oxidoreductases whose chief purpose is to reduce aldehydes and ketones to primary and secondary alcohols
<b>CuPep-2</b>	3.51	0.1	3.422	0.142	V. Good	Retrotransposon protein, DNA integration, protein transporter activity and protein import into nucleus, docking

FDR. The false discovery rate of a set of predictions is the expected percent of false predictions in the set of predictions. XCorr. The XCorr value is the cross-correlation value from the research. DelCn is the Delta Correlation value, which measures how different the first hit is from the second hit in the search results

A	B
1 MGLLDVGEK KSVIPRRVQR RWEKSTIFLS PSTQNRLONE	1 MESHISTFSH FRNRFLACDS VRAANPETLN RDGLVGLPSS
41 RRRKSTEREG EEEIRVAVCP <b><u>PSPSPLPPP VLPRRSVAVA</u></b>	41 AVKTNQECCR <b><u>SSPSRTFHHG GSLISRCTTA AGVFSGVILR</u></b>
81 <b><u>FCDAGSLKSF</u></b> VTPRNKSLLR RAQRALGIFF PSL	81 KISMEEANEI LKNKRIEGIS WLCSLSESEL DLLISIKMLV
	121 LQRAKAVGHE NLAEKFDLKM QRAIGFVLME HLKGLERTSD
	161 VSDLSQSALN ACNLLDSNLE KILSIDEIMA SICSDRRKKP
	201 GKRPQEKVDS RYKKNDKL

Fig. 1. The primary structure of Cupep-1 and Cupep-2 deduced from the proteome sequence of *Cucumis sativus* L. The amino acid sequence of protein precursors of Cupep-1 peptide (A) comprise of 113 amino acids. The cleaved peptide sequence of Cupep-1 is 35 amino acids (bold and underlined) are located at the N terminal. (B) The primary structure of Cupep-2. The protein precursors of Cupep-2 peptide comprise of 216 amino acids. The cleaved peptide sequence of Cupep-2 are 31 amino acids (bold and underlined) are located at the C terminal

The Cupep-1 peptide exhibited strong antimicrobial activity against *B. subtilis*, *E. coli*, *P. aeruginosa*, *St. aureus*, *S. cerevisiae* and *C. albicans* (Table 3). However, the peptide did not show any growth inhibitory effect on the fungi *Aspergillus* spp. (Table 3). The different growth inhibitory patterns on diverse types of microorganisms are likely to be related to differences in cell wall structures and protein synthesis of yeast, fungus and bacteria (Rauha et al. 2000).

We observed a broad antimicrobial activity against *E. coli*, *P. aeruginosa*, *St. aureus*, *B. subtilis* and *C. albicans* were 16, 15, 15, 15, and 17  $\mu\text{g}/100\text{ ml}$  media. The variation in the effectiveness of Cupep-1 peptide against different strains may depend on the differences in the permeability of cell of those microbes (Dorman and Deans 2004, Ramadan and Asker 2012).

**Table 2. The physico-chemical characterization of Cupep-1 and 2.**

Peptide	No. of a.a	PI	H .Life H/Y/B	GRAVY	AMP	M/Ch-SP	SP
CuPep-1	35	7.32	100h/20m/10m	0.403	Yes	No	Yes
CuPep-2	31	5.91	1h/2m/2m	0.165	might be	No	Yes

a.a: Amino acide. pI: Isoelectric point. H life: Half life. H/Y/B: Human/yeast/pacteria peptide,. M/Ch-SP: Mitochondrial signal peptide or chloroplast signal peptide. SP: Signaling peptide.

**Table 3. Antimicrobial activity of different concentrations of Cupep-1**

Sample ( $\mu\text{g}$ )		Diameter of inhibition (mm)					
		Bacteria				Yeast	
		<i>B. subtilis</i>	<i>St. aureus</i>	<i>E. coli</i>	<i>P. auuginosa</i>	<i>S. cervisiae</i>	<i>C. albicans</i>
Peptide	5	00.0 $\pm$ 0.00	00.0 $\pm$ 0.00	00.0 $\pm$ 0.00	00.0 $\pm$ 0.00	00.0 $\pm$ 0.00	00.0 $\pm$ 0.00
	10	00.0 $\pm$ 0.00	00.0 $\pm$ 0.00	00.0 $\pm$ 0.00	00.0 $\pm$ 0.00	00.0 $\pm$ 0.00	08.1 $\pm$ 0.07
	20	07.6 $\pm$ 0.67	08.3 $\pm$ 0.88	06.5 $\pm$ 0.33	07.3 $\pm$ 0.07	07.0 $\pm$ 0.33	10.3 $\pm$ 0.33
	30	11.5 $\pm$ 0.88	12.0 $\pm$ 0.66	08.7 $\pm$ 0.66	09.0 $\pm$ 0.66	09.5 $\pm$ 0.66	15.2 $\pm$ 0.66
	40	12.3 $\pm$ 0.67	14.3 $\pm$ 0.88	11.3 $\pm$ 0.67	12.0 $\pm$ 0.88	11.5 $\pm$ 0.33	18.0 $\pm$ 0.33
	50	15.0 $\pm$ 0.88	15.6 $\pm$ 0.67	14.0 $\pm$ 0.88	14.6 $\pm$ 0.67	14.0 $\pm$ 0.67	20.0 $\pm$ 0.67
	60	17.2 $\pm$ 0.00	18.1 $\pm$ 0.00	17.0 $\pm$ 0.33	16.4 $\pm$ 0.66	16.7 $\pm$ 0.88	22.5 $\pm$ 0.88
Tetracycline	10	24.0 $\pm$ 0.30	24.7 $\pm$ 0.07	25.0 $\pm$ 0.09	26.0 $\pm$ 0.06	00.0 $\pm$ 0.00	00.0 $\pm$ 0.00
Fluconazol	10	00.0 $\pm$ 0.00	00.0 $\pm$ 0.00	00.0 $\pm$ 0.00	00.0 $\pm$ 0.00	24.0 $\pm$ 0.12	25.2 $\pm$ 0.09

Each value represents mean of sample  $\pm$  Sd for n = 3 disks. Diameter of inhibition zone was measured as the clear area centered on the agar well containing the sample, well with non-inhibition zone were recorded. Measurements were taken after 24 hrs incubation with yeast, 24 - 48 hrs with bacteria.

It was found that the peptide Cupep-1 had a drastic negative effect on the biosynthesis of total protein and total lipids in cells of *B. subtilis* (Figs 3, 4). This effect positively correlated with increasing the Cupep-1 concentrations and incubation period. On the other hand, the peptide had only a slight impact on the biosynthesis of acid soluble phosphorus (Fig. 5)



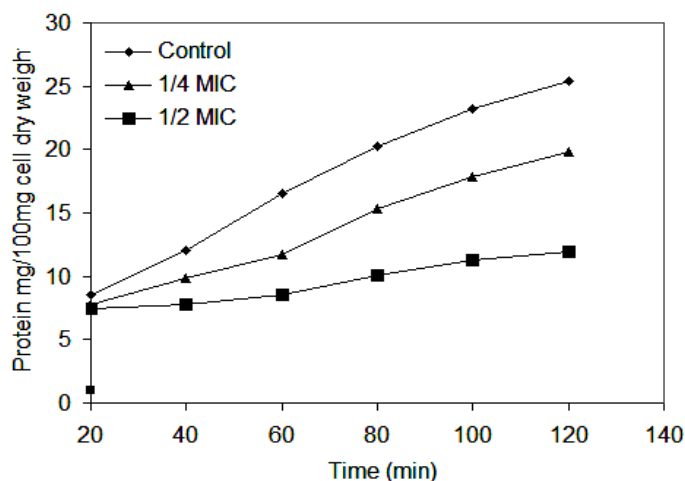


Fig. 3. Effect of Cupep-1 peptide on total protein content of *B. subtilis* NRRL B-94.

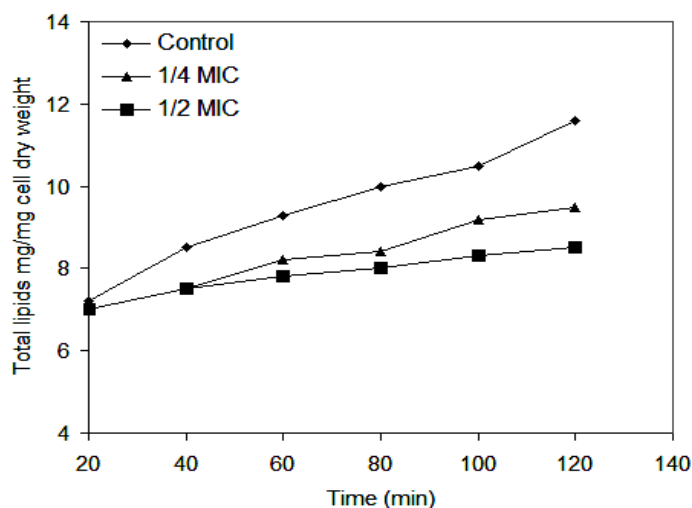


Fig. 4. Effect of Cupep-1 peptide on total lipid contents of *B. subtilis* NRRL B-94.

However, the peptide showed negative effects on total nucleic acids (RNA and DNA) in the cells of *B. subtilis* (Figs 6, 7). The effect of Cupep-1 resembles the action of some chemotherapeutic agents such as Mj-AMP1 and Mj-AMPS derived from purple jasmine (Cammue et al. 1992) that influence the biosynthesis of nucleic acids, or bind to nucleic acids to hinder transcription or translation. The majorities of these drugs are unselective and affects animal and bacterial cells alike, thereby their use is being limited (Ramadan and Asker 2012, Shuichi et al. 2000). It will be interesting to test the effect of Cupep-1 on animal cells to predict whether it can be used in human therapy.

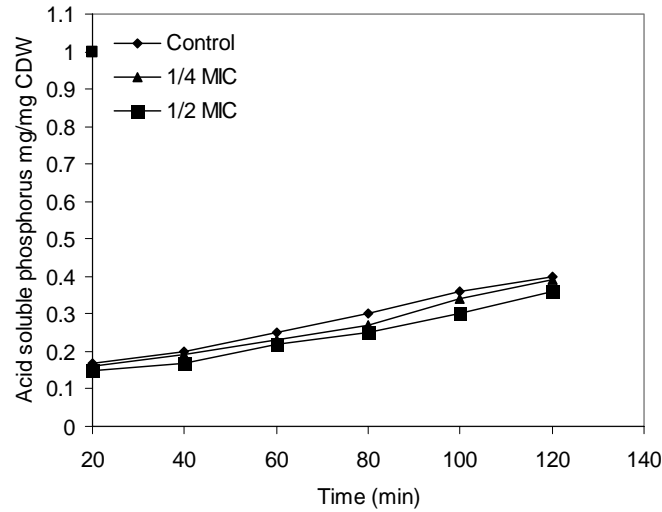


Fig. 5. Effect of Cupep-1 peptide on acid soluble phosphorus content of *B. subtilis* NRRL B-94.

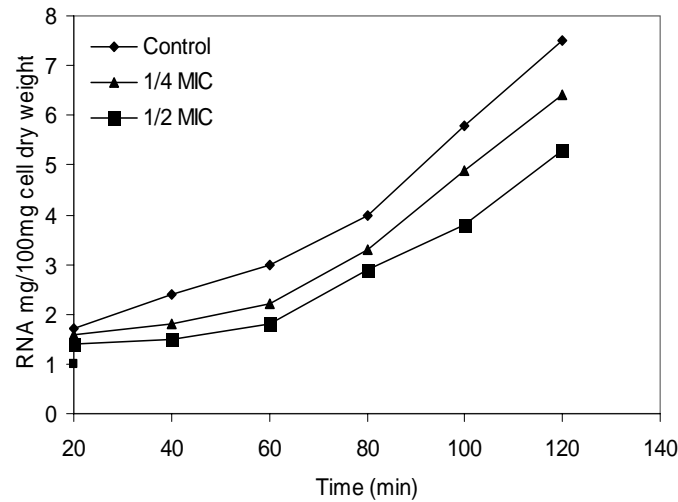


Fig. 6. Effect of Cupep-1 peptide on RNA content of *B. subtilis* NRRL B-94.

To examine the predicted function of Cupep-2 in plant signaling, a 4-week-old callus culture and propagated shoots were used to test the effect of Cupep-2 on globe artichoke plant cell development and long viability as it was found to have a short viable period (Shawky and Aly 2007). Four concentrations of synthetic Cupep-2 (25, 50 and 100 nM) were used to investigate the active concentration of the tested peptides in plant cell development. In the control

treatment, the excised shoots grow well for 4 weeks then started to senesce and finally died by two month (Fig. 8A). The shoots grown on 25 and 100 nM did not show any difference in comparison to the control shoots (Fig. 8A). In contrast, shoots grown on medium containing 50 nM of cupep-2 stayed green and did not show signs of senescence for up to two months (Fig. 8A). Interestingly, the shoots grown on 50 nM cupep-2 caused activation and initiation of terminal buds after six weeks of culture (Fig. 8B, C).

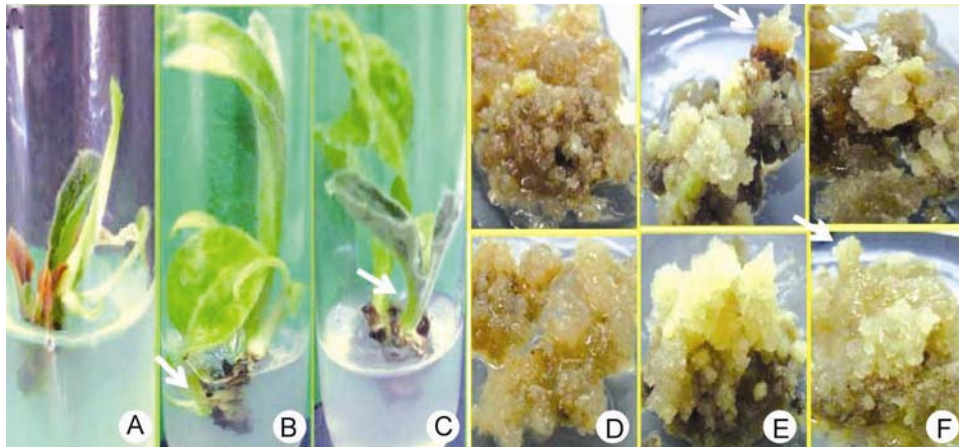


Fig. 6. Effect of different concentrations of the Cupep-2 peptide in shoot- and callus culture of globe artichoke (*Cynaracardunculus* var. *scolymus*) after two months of growth. (A) Control shoot without peptide, (B, C) shoots growing in the medium supplemented with 50 nM Cupep-2, (D) control callus, (E and F) callus growing in the media supplemented with 25 and 100 nM Cupep-2.

We further tested the impact of Cupep-2 on undifferentiated cells, callus of the globe artichoke. Calli were grown for two months on callus maintenance medium. At the end of the experiment, calli of the control treatment turned to brown color (Fig. 8D). Calli grown in the medium supplemented with 50 nM Cupep-2 did not trigger any development changes in undifferentiated cells and did not show a difference from the control (Fig. 8D). In contrast, the Cupep-2 concentrations 25 and 100 nM induced healthy creamy calli growth (Fig. 8E). In addition, the concentration 100 nM favored organogenesis leading to the development of somatic embryos (Fig. 8E). None of the concentrations showed inhibitory callus growth.

The globe artichoke cultures responded to the Cupep-2 peptide in a concentration and developmental stage dependent manners. They promoted organogenesis or shoot development. These results would assign Cupep-2 a signaling role in plant development. The Cupep-2 seems to play a similar role of

cytokinins in plants. It is well known that cytokinins stimulate plant cell division and participate in the induction of adventitious bud formation (Gaspar et al. 1996, Gaspar et al. 2003). Plant growth hormones are thought to be involved in differentiation and further development of the morphogenetic event (Becraft 1998), which is similar to what we have observed after the application of Cupep-2.

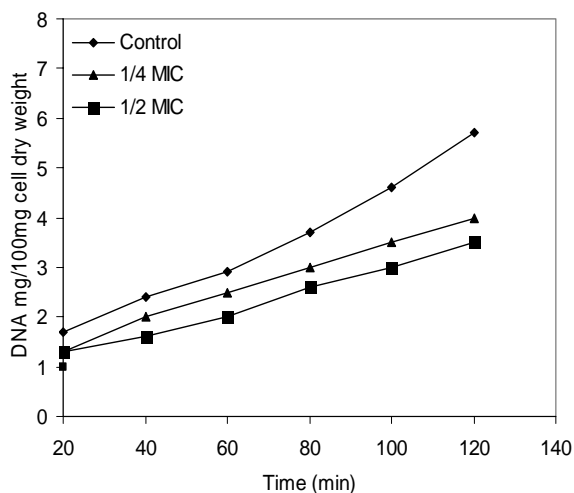


Fig. 7. Effect of Cupep-1 peptide on DNA content of *B. subtilis* NRRL B-94.

There is an increasing attention to and research on the role of small peptides in plant defence and development. It is evidently clear that the understanding and recognition of the exogenous peptides signals pathways was induced from the plant indigenous cellular signals component system. In this paper we try to examine the actual biological activities and confirmed the predicted function through analysis of two synthetic peptides, Cupep-1 and Cupep-2, isolated which were extracted and sequenced from Chinese Long cucumber phloem sap. The Cupep-1 peptide has shown promising antimicrobial activity against bacteria and yeast by affecting protein biosynthesis. This property would render Cupep-1a potential natural food preservative to inhibit microbial growth. The peptide Cupep-2 showed a potential role in plant development. The effect of Cupep-2 on cell or organ differentiation was dependent on the concentration and the developmental stage of the treated cells or tissue. Obviously, we are just beginning to unravel the actual biological function of these two peptides in the phloem sap of Chinese cucumber. More experiments are needed to identify a new regulatory signaling peptide of plant defense, growth promotion and development that lead to new discoveries in signal perception and cellular responses.

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