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A novel synthetic peptide from a tomato defensin exhibits antibacterial activities against *Helicobacter pylori*

M. M. Rigano,[a] A. Romanelli,[b] A. Fulgione,[a] N. Nocerino,[a] N. D'Agostino,[c] C. Avitabile,[b] L. Frusciante,[d] A. Barone,[a] F. Capuano[e] and R. Capparelli[a]*

Defensins are a class of cysteine-rich proteins, which exert broad spectrum antimicrobial activity. In this work, we used a bioinformatic approach to identify putative defensins in the tomato genome. Fifteen proteins had a mature peptide that includes the well-conserved tetradisulfide array. We selected a representative member of the tomato defensin family; we chemically synthesized its γ-motif and tested its antimicrobial activity. Here, we demonstrate that the synthetic peptide exhibits potent antibacterial activity against Gram-positive bacteria, such as *Staphylococcus aureus* A170, *Staphylococcus epidermidis*, and *Listeria monocytogenes*, and Gram-negative bacteria, including *Salmonella enterica* serovar Paratyphi, *Escherichia coli*, and *Helicobacter pylori*. In addition, the synthetic peptide shows minimal (<5%) hemolytic activity and absence of cytotoxic effects against THP-1 cells. Finally, SolyC exerts an anti-inflammatory activity *in vitro*, as it downregulates the level of the proinflammatory cytokines TNF-α and IFN-γ. Copyright © 2012 European Peptide Society and John Wiley & Sons, Ltd.

Supporting information may be found in the online version of this article.

Keywords: antimicrobial peptide; defensin; tomato; *Helicobacter pylori*

Introduction

Attention towards new antimicrobial agents is growing because of the rising of antibiotic and multidrug bacterial resistance [1,2]. Particular interest is devoted to the development of novel antibiotics against *Helicobacter pylori*, a Gram-negative bacterium that chronically infects the gastric mucosa of more than half of the human population and sometimes causes severe diseases, such as gastric cancer [3]. *H. pylori* LPS shows extremely low endotoxic activity, compared with typical Gram-negative LPSs, allowing it to establish chronic colonization without causing a systemic inflammatory response [4]. Currently, the prevalent approach for *H. pylori* eradication is based on antibiotic treatment. However, antibiotics cause serious effects on the intestinal microflora and induce antibiotic-resistant strains [5].

Antimicrobial peptides are cationic molecules of the innate immune system and represent a valid defense mechanism against infections because of their broad spectrum antibiotic activity and low eukaryotic cell toxicity. In addition, they rarely induce bacterial resistance [1,6]. Defensins are the only class of peptides in the innate immune response that is conserved among plants, invertebrates, and vertebrates [7]. They are cysteine-rich proteins with a common three-dimensional structure rich in β-sheets [1]. Plant defensins (originally classified as γ-thionins, [8]) are small, basic, highly stable proteins with antifungal and antibacterial properties [8,9]. Their structure resembles that of insect and mammalian defensins, corroborating the idea that all defensins evolved from a single precursor [8]. Indeed, Yount and Yeamen [10] identified a conserved γ-core motif ($GXCX_{3\text{-}9}C$) – composed of two antiparallel β-sheets and an interposed loop [10] – in disulphide-containing AMPs from several phylogenetically diverse organisms. This motif has a net cationic charge and can be found in other host-defense polypeptides with antimicrobial activity, such as venoms, toxins, or microbicidal chemokines [11].

The recent release of the tomato genome sequence [12] facilitates the identification of genes encoding proteins with potential antimicrobial activity. In this work, we used bioinformatics methods to identify and characterize tomato defensins. Then, on the basis of sequence information, we selected a representative member of the family; we chemically synthesized a peptide (SolyC) corresponding

* *Correspondence to: Rosanna Capparelli, University of Naples 'Federico II', School of Biotechnological Sciences, Department of Soil, Plant, Environmental and Animal Production Sciences, Via Università 100, 80055 Portici, Naples, Italy. E-mail: capparel@unina.it*

a *University of Naples 'Federico II', School of Biotechnological Sciences, Department of Soil, Plant, Environmental and Animal Production Sciences, Via Università 100, 80055, Portici, Italy*

b *University of Naples 'Federico II', School of Biotechnological Sciences, Department of Biological Sciences, Via Mezzocannone 16, 80134, Naples, Italy*

c *CRA-ORT, Agricultural Research Council, Research Centre for Vegetable Crops, Via Cavalleggeri 25, 84098, Pontecagnano, SA, Italy*

d *University of Naples 'Federico II', Department of Soil, Plant, Environmental and Animal Production Sciences, Via Università 100, 80055, Portici, Italy*

e *Department of Food Inspection IZS ME, via Salute 2, 80055, Portici, Italy*

Abbreviations used: *LPS, lipopolysaccharide; AMP, antimicrobial peptides; ASA, acetylsalicylic acid; MIC, minimum inhibitory concentration.*

to its γ-motif and finally tested its antimicrobial activity. We demonstrate that SolyC has antibacterial activity against a panel of human pathogens, including *H. pylori*, and displays anti-inflammatory activity *in vitro*.

Material and Methods

Bioinformatics Analysis

HMM profile PF00304 was retrieved from Pfam [13]. The *hmmsearch* program (e-value $1e^{-5}$; http://hmmer.org) was used to search against tomato proteins (iTAG v.2.3). Further, 57 plant defensins were retrieved from PhytAMP [14].

Multiple protein sequence alignments were generated using ClustalW [15]. Sequence distances were calculated with PROTDIST using the Dayhoff PAM matrix, and neighbor-joining trees were built using NEIGHBOR (from Phylip v3.67; http://evolution.genetics.washington.edu/phylip.html). Unrooted trees were displayed with FigTree (v.1.3.1; http://tree.bio.ed.ac.uk/software/ figtree/).

The overall charge of the γ-core motifs and of the synthetic peptide was estimated at pH 7 using Biochemistry online (http://vitalonic.narod.ru/biochem).

Peptide Synthesis

The amino acids used for the peptide synthesis Fmoc-Phe-OH, Fmoc-Ser(OtBu)-OH, Fmoc-Gly-OH, Fmoc-Asn(Trt)-OH, Fmoc-Cys (Trt)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Thr(OtBu), Fmoc-Lys(Boc)-OH, the Rink amide MBHA, and the activators *N*-hydroxybenzotriazole (HOBT) and *O*-benzotriazole-*N*,*N*,*N*′,*N*′-tetramethyl-uronium-hexafluoro-phosphate (HBTU) were purchased from Novabiochem. Acetonitrile (ACN) was from Reidel-deHaën and dry *N*,*N*-dimethyl-formamide (DMF) from LabScan. All other reagents were from Sigma Aldrich. LC-MS analyses were performed on an LC-MS Thermo Finnigan with an electrospray source (MSQ) on a Phenomenex Jupiter 5 μ C18 300 Å, (150 × 4.6 mm) column. Purification was carried out on a Phenomenex Jupiter 10 μ Proteo 90 Å (250 × 10 mm) column. The Peptide SolyC (FSGGNCRGFRRRCFCTK-NH_2) was synthesized on solid phase by Fmoc chemistry on the MBHA (0.54 mmol/g) resin by consecutive deprotection, coupling, and capping cycles [16]. Deprotection: 30% piperidine in DMF, 5 min (2×). Coupling: 2.5 equivalents of amino acid + 2.49 equivalents of HOBT/HBTU (0.45 M in DMF) + 3.5 equivalents NMM, 40 min. Capping: acetic anhydride/DIPEA/DMF 15/15/70 v/v/v, 5 min. The peptide was cleaved off the resin and deprotected by treatment of the resin with a solution of TFA/TIS/H_2O 95/2.5/2.5 v/v/v, 90 min. TFA was concentrated, and peptides were precipitated in cold ethylic ether. Analysis of the crudes was performed by LC-MS using a gradient of ACN (0.1% TFA) in water (0.1% TFA) from 5% to 70% in 30 min. Purification was performed by semipreparative RP-HPLC using a gradient of ACN (0.1% TFA) in water (0.1% TFA) from 5% to 70% in 30 min.

The identity of the peptide SolyC (FSGGNCRGFRRRCFCTK-NH_2) was verified by mass spectrometry.

Calculated mass (Da): 1994.33, $[M+2H]2^+$: 998.16; $[M+3H\,3^+]$: 665.77; found (Da): $[M+H]^+$ 1995.49; $[M+2H]2^+$: 997.75; $[M+3H\,3^+]$: 665.77.

Bacteria

List and origin of the different strains used in this study, as well as their resistance/susceptibility to conventional antibiotics are reported in Tables S1 and S2. Bacterial isolates were from patients hospitalized at the Medical School of the University of Naples 'Federico II' and at the 'Villa Betania' hospital (Naples, Italy). Species identification was carried out by PCR [17–21]. Bacteria were grown at 37 °C in TSB or LB medium, harvested in the exponential phase (OD 600 nm 0.6–0.8), centrifuged (10 min at 8×10^3 g) and resuspended in Muller Hinton broth at the concentration of ~10^5 CFU/ml. The antibiotic-susceptibility profile of strains was determined using the disk diffusion method on Mueller-Hinton agar, according to the NCCLS guidelines (2002). The antibiotics used and their concentrations were as follows: trimethropim + sulfamethoxazole (25 μg; SXT), sulphonamide (300 μg; S3, SUL), nalidixic acid (30 μg; NA), enrofloxacin (10 μg; ENX), ciprofloxacin (5 μg; CIP), ampicillin (10 μg; AMP), cefalotin (30 μg; KF, CF), tetracycline (30 μg; TE, TET), gentamicin (10 μg; CN, GEN), kanamycin (30 μg; K,KAN), ceftazidime (30 μg; CAZ), streptomycin (10 μg; S, STR), chloramphenicol (30 μg; C, CLO), amoxicillin + clavulamic acid (30 μg; AMC), cefoxitin (30 μg; FOX). All antibiotics were provided by OXOID and Becton Dickinson.

Antibacterial and Hemolytic Activity

Bacteria were distributed in triplicate into plates (60 μl/well), mixed with SolyC dilutions (5–100 μg/ml; 40 μl/well) and incubated at 37 °C for 20 h. The minimal concentration of SolyC causing 100% growth inhibition (MIC_{100}) was determined by measuring the absorbance at 600 nm (Biorad microplate reader model 680, CA). The antibacterial activity was measured by spotting 10 μl from each well on TSA or LB agar and counting the CFUs [22]. The antibacterial test was extended to the probiotic bacteria *Lactobacillus plantarum* and *Lactobacillus paracasei*. The test was performed in triplicate. SolyC was tested for its hemolytic activity using mouse red blood cells. The hemolytic activity was measured according to the formula $OD_{peptide} - OD_{negative\ control}/OD_{positive\ control} - OD_{negative\ control} \times 100$ where the negative control (0% hemolysis) was represented by erythrocytes suspended in saline and the positive control (100% hemolysis) was represented by the erythrocytes lysed with 1% triton X100 [22].

The LC50 value relative to the SolyC was calculated as described [23].

Cell Culture

The THP-1 human acute monocytic leukemia cells (American Tissue Culture Collection, MD, USA) were cultured in complete medium (CM) consisting of RPMI medium (Gibco, Scotland), 10% fetal bovine serum, 100 IU/ml penicillin, and 100 μg/ml streptomycin (all from Gibco). Cell adhesion was induced with phorbol myristate acetate (2 μg/ml/well).

Cell Viability

Trypan blue test

THP-1 cells (10^6 cells/well) were let adhere (37 °C, 5% CO_2) in CM. Then, they were incubated first with SolyC (60–120 μg/ml for 24, 48, or 72 h), and then with 1% trypsin (1.5 ml/well at 37 °C for 3 min) and finally with CM (3 ml/well). The whole mixture was transferred into a test tube and centrifuged (3 min at 1000 g). The pellet was resuspended in 1 ml CM. A 10 μl of cell suspension was mixed with 10 μl of Trypan blue, and the percentage of viability was determined using the formula: N° viable cells/(N° non viable cells + viable cells) × 100.

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MTT

Cell viability was determined by the CellTiter 96® AQueous One Solution Cell Proliferation Assay (MTS) (Promega, WI, USA). THP-1 cells (2500/well) were incubated at 37 °C in 5% CO_2. SolyC (60 μg/ml and 120 μg/ml) or PBS was added to the medium after cell adhesion. At each time point, MTS solution (20 μl/well) was added. Absorbance was recorded at 490 nm after 2 h using an EnVision 2102 multilabel reader (PerkinElmer, USA).

Nitrite Formation in THP-1 Cells

THP-1 cells adhesion (10^6/well) was induced with phorbol myristate acetate (2 μg/ml/well; 12 h) and then stimulated for 24, 48, or 72 h with LPS (10 μg/ml), SolyC (50 μg/ml), or with a combination of both. Nitrite accumulation (NO_2^-, μmol/10^6 cells) in the medium was determined by the Griess reaction [24].

ELISA Test of Proinflammatory Cytokines

TNF-α and IFN-γ levels were estimated by the sandwich ELISA assay. Briefly, THP-1 cells (10^6 cells/well) were stimulated with LPS (10 μg/ml; 1 h), treated with 50 μg/ml ASA or SolyC (50, 100, or 120 μg/ml; 1 h) in the presence or absence of LPS (10 μg/ml). The supernatants from these cells (100 μl/well) were transferred into the wells of a plate previously coated with mouse anti-human TNF-α (BD Pharmingen; 50 μl diluted 2×10^{-3}/well) or mouse anti-human IFN-γ (Biosciences, 50 μl diluted 2×10^{-3}/well) along with a second dose of anti-IFN-γ or TNF-α, HRP-labeled rabbit anti mouse IgG diluted 10^{-3} (100 μl/well) and TMB peroxidase substrate (BIORAD; 100 μl/well), in the order. The optical density of each well was read at 405 nm using a microplate reader (Bio-Rad, Japan). Triplicate positive and negative controls were included in each plate [25].

Ethical Treatment

The study investigated *in vitro* the antibacterial activity of a synthetic peptide on *H. pylori* isolates provided by 'Villa Betania' hospital (Naples, Italy). The study neither investigate clinical aspects of the disease nor it uses human specimen. The study therefore does not require the Ethic Committee approval.

Results

Bioinformatics Analysis

A total of 16 defensin proteins were identified by using the Pfam HMM profile PF00304 (Table 1). An additional protein (tagged with * in Table 1) was initially included in this dataset based on the iTAG functional annotation [12].

All proteins but two have a mature peptide that includes the eight conserved cysteines involved in disulfide bonds essential for structural folding [8]. The most represented consensus sequence is C–X_{10}–C–X_5–C–X_3–C–X_9–C–X_6–C–X–C–X_3–C, present in 11 out of 15 proteins. The spacing of cysteines is different in some instances. By contrast, the Solyc07g016120 and Solyc11g028060 proteins lack the tetradisulfide array (Table 1) and were excluded from the subsequent phylogenetic analyses. We assigned 12 proteins to the class I of plant defensins, characterized by an endoplasmic reticulum signal sequence and a mature defensin domain. The remaining three proteins were assigned to the class II of plant defensins, characterized by the presence of an additional C-terminal prodomain (Table 1; [26]). With the exception of Solyc11g028060 and Solyc07g007760, which consist of one and three exons, respectively, nearly all the identified γ-thionins are composed of two exons. Finally, we investigated the chromosomal localization of these genes: three are on chromosomes 4, eight on chromosome 7, five on chromosome 11, and one on chromosome 9. Also, we identified a cluster of five members on chromosome 7 and a cluster of three members on chromosome 11.

The 15 mature defensin peptides were used to generate the multiple sequence alignment shown in Figure 1A. Tomato defensins exhibit clear sequence conservation, just like plant γ-thionins. Importantly, the γ-core motifs differ among tomato defensins in their primary amino acid sequences, even if distinct groups of γ-core motifs can be clearly distinguished based on sequence similarity (Figure 1A).

To show the phylogenetic relationships within the tomato defensin family, an unrooted neighbor-joining tree was built (Figure 1B). Two distinct clades were clearly visible. The first one includes class II defensins, whereas the second one includes class I γ-thionins. This clade can be further divided into four subclades. The largest subclade includes five members, four of which belong to the gene cluster identified on chromosome 7. An additional neighbor-joining tree included all the plant defensins collected from the PhytAMP database ([14]; supplementary figure 1). The clustering of class I and class II defensins was still clearly observable. Indeed, class II tomato defensins are grouped with further proteins from *Petunia hybrida* and *Nicotiana* confirming that these defensins are typical of the Solanaceae family [26]. Solyc07g007760 was selected as representative of the tomato defensin family because the primary sequence of its γ-core motif is almost identical to that of five more tomato defensins, and it displays features compatible with antimicrobial activity, such as its total net charge, which is +5 (Figure 1A). A 17 amino acid long peptide (highlighted in Figure 1A) containing the γ-core motif sequence of the defensin Solyc07g007760 was chemically synthesized and tested for antibacterial activity.

Characterization of Bacterial Strains

The different bacteria strains used in this study were characterized by phenotypical (antibiotic resistance/susceptibility patter) analysis. Results of antibiotic resistance are reported in Table S2. All *H. pylori* strains were resistant to ampicillin (10 μg; AMP), gentamicin (10 μg; CN, GEN), kanamycin (30 μg; K,KAN), streptomycin (10 μg; S, STR), and amoxicillin + clavulamic acid (30 μg; AMC) and sensitive to the remaining antibiotics tested. *Staphylococcus aureus* A170 and *Staphylococcus epidermidis* were sensitive to all antibiotics except to nalidixic acid (30 μg; NA). *Salmonella enterica* serovar Paratyphi was resistant to tetracycline (30 μg; TE, TET) and sensitive to the other antibiotics, whereas the remaining strains were sensitive to all antibiotics.

Antibacterial and Anti-inflammatory Activity

The synthetic peptide SolyC showed antimicrobial activity against Gram-negative bacteria, including *Helicobacter pylori*, at low concentration (MIC: 15 μg/ml) and, at higher concentration (MIC: 40 μg/ml), also against Gram-positive bacteria (Table 2). In addition, SolyC displayed very low antibacterial activity (much lower than that of gentamicin) against probiotic bacteria (*L. plantarum* and *L. paracasei*) (Table 3). The synthetic peptide, at 50 μg/ml, displayed

Table 1. List of tomato defensins[a]

gene id	Chromosome	gene coordinates	Num. exons	protein length	class	spacing of cysteine residues
Solyc04g008470	4	2087094-2089108	2	73	I	C-X_{10}-C-X_5-C-X_3-C-X_9-C-X_6-C-X-C-X_3-C
Solyc04g009590	4	2983229-2984644	2	76	I	C-X_{10}-C-X_5-C-X_3-C-X_9-C-X_8-C-X-C-X_3-C
Solyc04g072470	4	57080225-570813	2	80	I	C-X_{10}-C-X_5-C-X_3-C-X_9-C-X_7-C-X-C-X_3-C
Solyc07g006380	7	1207489-1208297	2	105	II	C-X_{10}-C-X_5-C-X_3-C-X_9-C-X_6-C-X-C-X_3-C
Solyc07g007710	7	2362299-2363266	2	76	I	C-X_{10}-C-X_5-C-X_3-C-X_9-C-X_6-C-X-C-X_3-C
Solyc07g007730	7	2374606-2376176	2	79	I	C-X_{10}-C-X_5-C-X_3-C-X_9-C-X_6-C-X-C-X_3-C
Solyc07g007740	7	2383190-2383538	2	76	I	C-X_{10}-C-X_4-C-X_3-C-X_9-C-X_8-C-X-C-X_3-C
Solyc07g007750	7	2390188-2391454	2	75	I	C-X_{10}-C-X_5-C-X_3-C-X_9-C-X_6-C-X-C-X_3-C
Solyc07g007760	7	2394467-2396320	3	78	I	C-X_{10}-C-X_5-C-X_3-C-X_9-C-X_6-C-X-C-X_3-C
Solyc07g009260	7	4313698-4315233	2	76	I	C-X_{10}-C-X_4-C-X_3-C-X_9-C-X_8-C-X-C-X_3-C
Solyc07g016120	7	6304496-6305153	2	88	n.d.	C-X_{10}-C-X_5-C-X_3-C
Solyc09g074440	9	61747475-617482	2	74	I	C-X_{10}-C-X_5-C-X_3-C-X_9-C-X_6-C-X-C-X_3-C
Solyc11g006260	11	1008356-1008972	2	76	I	C-X_{10}-C-X_5-C-X_3-C-X_9-C-X_6-C-X-C-X_3-C
Solyc11g006950	11	1452336-1452920	2	73	I	C-X_{10}-C-X_5-C-X_3-C-X_9-C-X_6-C-X-C-X_3-C
Solyc11g028040	11	16598859-16599530	2	105	II	C-X_{10}-C-X_5-C-X_3-C-X_9-C-X_6-C-X-C-X_3-C
Solyc11g028060*	11	16615578-16615835	1	85	n.d.	C-X_6-C-X-C-X_3-C
Solyc11g028070	11	16643406-16644076	2	105	II	C-X_{10}-C-X_5-C-X_3-C-X_9-C-X_6-C-X-C-X_3-C

[a]This table shows, for each sequence, chromosomal localization, gene coordinates, number of exons, protein length, class, and the consensus sequence describing the spacing of cysteines. Gray rows indicate proteins that lack the tetradisulfide array. '*' identified based only on the iTAG functional annotation. n.d., not determined.

<5% hemolytic activity, and the LC50 value was 0.27 mg/ml. In addition, at 60–120 μg/mL, displayed no cytotoxic effects, SolyC-treated THP-1 cells remained viable for up to 72 h (Figure 2A and B). Furthermore, SolyC reduced ($p < 0.01$) the production of NO_2^- by cells stimulated with LPS, compared with control cells (stimulated with LPS, but not treated with SolyC) (Table 4).

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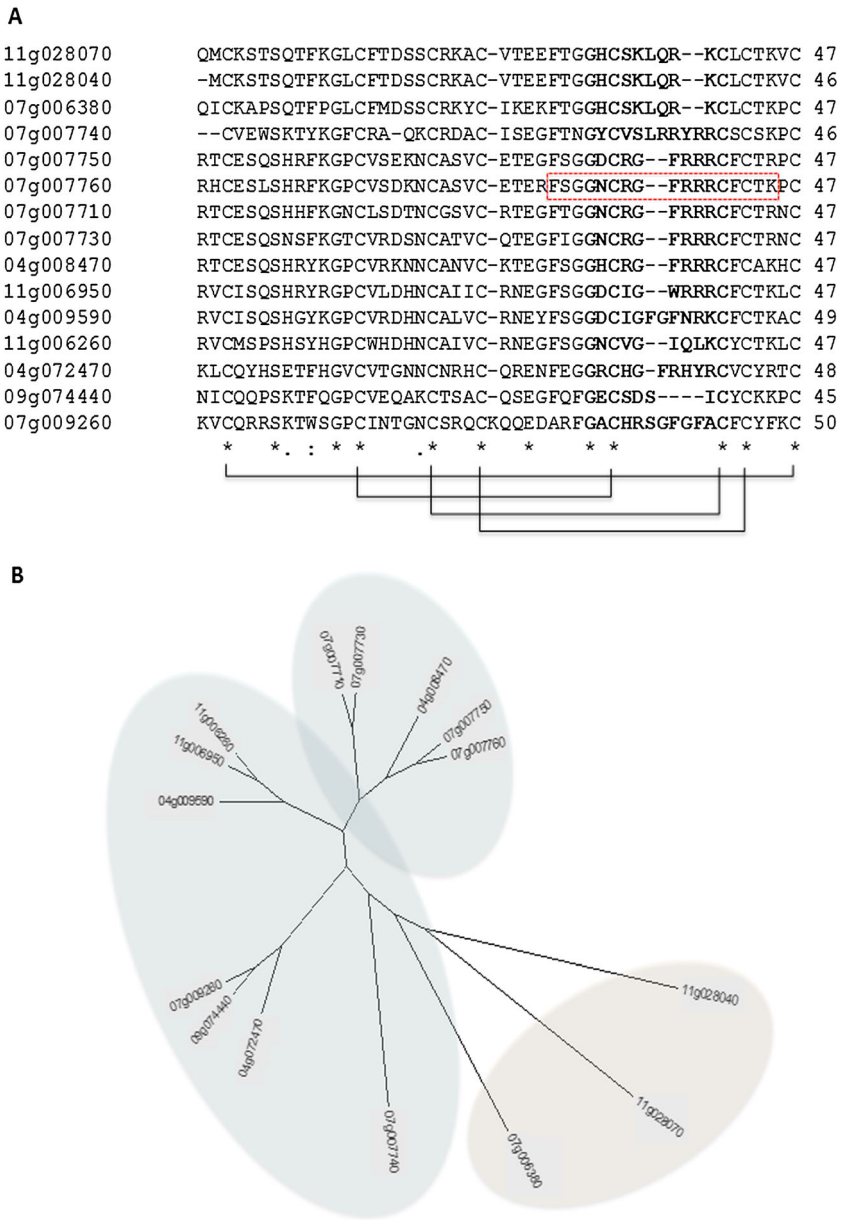

Figure 1. (A) Multiple alignment of the 15 tomato mature defensin proteins. Disulfide bonds between the eight conserved cysteines are shown by connecting lines. Gamma-core motifs are shown in bold. The sequence of the synthetic peptide SolyC is highlighted in the box. (B) The alignment in (A) was used to generate the phylogenetic tree for the tomato defensin family.

THP-1 cells, stimulated with LPS and then challenged with ASA or SolyC, showed significantly lower levels of TNF-α and IFN-γ, compared with cells treated with LPS. The results show that both SolyC and ASA curb the synthesis of the proinflammatory cytokines TNF-α and IFN-γ (Figure 3A and B). In the absence of the agent causing inflammation (LPS), SolyC or ASA does not induce inflammation (Figure 3A and B). These experiments demonstrate that SolyC exerts anti-inflammatory activity.

Discussion

In this paper, we investigated the antimicrobial and anti-inflammatory activity of a synthetic peptide derived from the tomato defensin family. Plant defensins are appropriate candidates for therapeutic applications because of their broad range of antimicrobial activity, their stability, and low cytotoxicity in humans [9].

We are aware that via Hidden Markov Model searches, a genome wide search of defensin-like genes is possible [27]. However, our aim was to identify a reliable defensin core gene set rather than detecting all the possible defensin-like genes present in the tomato genome. We identified 17 tomato defensins, which are composed of two exons and one intron of variable size. As in the case of plant defensins, the first exon almost entirely encodes the signal peptide, whereas the second encodes the central defensin domain [6]. Then, we grouped the tomato defensins according to their class membership. It is well documented that plant defensins can be divided into two classes and that defensins of class II are limited to solanaceous plants [26]. As a member of the Solanaceae, tomato has defensins

Table 2. List of the bacteria strains used in this study and SolyC MIC for each strain

Bacterial species/strains	SolyC MIC (μg/ml)
Gram +	
Staphylococcus aureus A170	40
Staphylococcus epidermidis	40
Listeria monocytogenes	40
Gram −	
Salmonella enterica serovar Paratyphi	15
Escherichia coli	15
Helicobacter pylori VB*1	15
Helicobacter pylori VB*2	10
Helicobacter pylori VB*3	15
Helicobacter pylori VB*4	12
Helicobacter pylori VB*5	15
Helicobacter pylori VB*6	10
Helicobacter pylori VB*7	15
Helicobacter pylori VB*8	10
Helicobacter pylori VB*9	10
Helicobacter pylori VB*10	15

* Villa Betania hospital.

Table 3. Antimicrobial activity of SolyC and Gentamicin on probiotic bacteria

	SolyC[a] 50 μg/ml	Gentamicin[a] 5 μg/ml
Lactobacillum plantarum	15% ± 2	97% ± 4
Lactobacillum paracasei	13% ± 2	96% ± 3

[a]Data are reported as percentage of bacterial growth inhibition ± standard deviation.

belonging to both classes (Table 1). Finally, a genome overview allowed two defensin gene clusters to be identified. Defensin gene clusters have been already observed in Arabidopsis, and it has been assumed that individual clusters have evolved through local duplications [27]. The same mechanism very likely caused the expansion of tomato defensin gene family.

In this work, we were interested in the identification and synthesis of novel peptides active against human pathogens. By sequence alignment with known γ-motifs, which are recognized to be the major determinants of the antimicrobial activity of several peptides produced by organism belonging to all kingdoms of life, we identified in the defensin Solyc07g007760 its putative γ-motif [28].

It is known that plant defensins are mainly active against fungal pathogens and, less frequently, against Gram-positive bacteria [6]. In this study, we showed that SolyC controls the bacterial load and, surprisingly, especially the growth of the Gram-negative

Table 4. NO_2^- production of THP-1 cells subjected to four treatment protocols

Treatment	NO_2^- production[a]		
	24 h[b]	48 h[b]	72 h[b]
No treatment	0.118 ± 0.05**	0.254 ± 0.03**	0.557 ± 0.01**
SolyC	0.118 ± 0.02**	0.277 ± 0.03**	0.55 ± 0.03**
LPS	1.938 ± 0.2	2.75 ± 0.4	4.09 ± 0.3
LPS + SolyC	0.59 ± 0.05**	0.925 ± 0.03**	1.332 ± 0.1**

[a]Data are expressed as micromoles of NO_2^- for 10^6 input cells and are means ± standard deviation of three different experiments each performed in triplicate.

[b]Time of incubation.

** $p < 0.01$ *versus* LPS according to Student's *t* test.

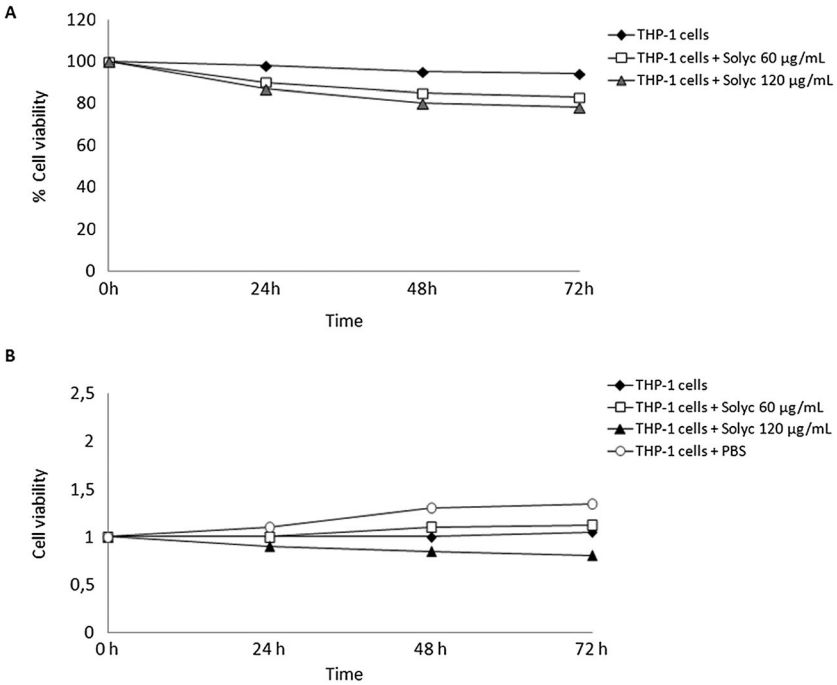

Figure 2. Analysis of cell viability. (A) THP-1 cells were treated with SolyC, and cell viability was determined by Trypan blue test. (B) THP-1 cells were treated with SolyC or PBS, and cell viability was determined by MTT assay.

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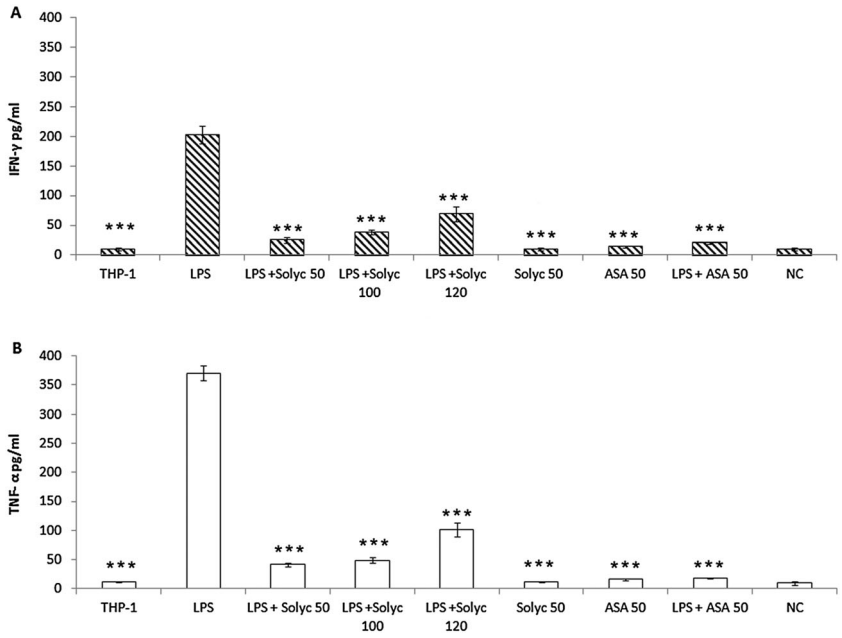

Figure 3. Anti-inflammatory activity. The levels of IFN-γ (A) and TNF-α (B) were determined by a sandwich ELISA test in THP-1 cells untreated; THP-1 cells stimulated with LPS for 1 h and then treated with SolyC (50, 100, or 120 μg/ml); THP-1 cells treated with Solyc (50 μg/ml) or ASA (50 μg/ml) for1 h; THP-1 cell stimulated with LPS for 1 h and then treated with ASA (50 μg/ml). Negative Control (NC): culture medium RPMI. Results from two representative experiments are presented as mean value ± SD. Statistical analysis was performed by Student's *t* test, ***$p < 0.001$.

bacterium *H. pylori*. This could be probably due to a strong electrostatic interaction between the cationic peptide SolyC and the anionic bacterial membranes. Work is under way to elucidate the interaction of the tomato peptide with the bacterial membrane and to determine the relative contribution of other residues on the antibacterial potency of this peptide.

In addition, we demonstrated that SolyC downregulates the level of proinflammatory cytokines and that this effect is comparable with that of ASA, a well-known anti-inflammatory drug. It is reported that human defensins in mixture with microbial antigens attenuate proinflammatory cytokine responses by dendritic cells in culture and attenuate proinflammatory cytokine responses in the nasal fluids of exposed mice [29]. The exact mechanisms are unknown; however, defensins first start by binding to microbial products attenuating inflammatory-inducing capacity. Here, we showed that also a synthetic peptide comprising the γ-motif of a plant defensin exerts an anti-inflammatory activity *in vitro*.

Moreover, we determined to what extent SolyC spared probiotic bacterial species, considering that intestinal flora represents a defense barrier against pathogens [30]. Whereas gentamicin killed the totality of the probiotics tested (see methods), SolyC killed a minority of each bacterial species (Table 3). In addition, we observed a general lack of human red blood cells hemolysis, the nontoxicity of SolyC towards eukaryotic cells *in vitro* and reduced synthesis of NO_2^- in cells treated with LPS. These additional properties make SolyC a feasible candidate as a new generation drug.

In conclusion, the results from this study suggest an analogy between endogenous AMPs and SolyC, a peptide of plant origin. Both display a twofold role, rapidly acting against pathogens and reducing inflammation. These findings demonstrate how the γ-core of plant defensins represents a potential source of antimicrobial molecules and may provide new opportunities in the field of therapeutic drug design and of plant biotechnology.

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