Sustainable Agriculture Research; Vol. 1, No. 2; 2012
ISSN 1927-050X E-ISSN 1927-0518
Published by Canadian Center of Science and Education

Activity of Naturally Derived Antimicrobial Peptides against Filamentous Fungi Relevant for Agriculture

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Received: June l2, 2012 Accepted: July 10, 2012 Online Published: July 23, 2012

doi:10.5539/sar.v1n2p211 URL: http://dx.doi.org/10.5539/sar.v1n2p211

Abstract

The search for environmentally biocompatible and cost-effective methods to control filamentous fungi in agriculture is becoming increasingly urgent. *In vitro* antimicrobial activity of three synthetic peptides was investigated against some filamentous fungi with agricultural relevance. The peptides were an analog of Temporin called Temporizina, a fragment from Pleurocidin termed Plc-2, and a peptide identified from sesame seeds named Pses3. Antimicrobial activity of these peptides towards filamentous fungi has not been previously reported. Seven plant pathogenic or mycotoxigenic fungal species, isolated from plant tissues were assayed: *Alternaria solani*, *Colletotrichum gloesporioides*, *Fulvia fulvum*, *Fusarium oxisporum*, *Aspergillus niger*, *A. ochraceus* and *Penicillium digitatum*. Values of Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) were determined and compared with the commercially available fungicide Captan as a positive control. The peptides showing greatest inhibition were Pses3 and Plc-2 and *C. gloesporioides* was the most sensitive of the evaluated fungi. The MIC values for Plc-2 and Pses3 peptides ranged from 0.64 μM to 10.25 μM. These values were much lower than those observed for Captan, suggesting the potential of these peptides as fungicides. In particular, Pses3 is a novel peptide derived from sesame seeds not reported in databases.

Keywords: antimicrobial peptides, temporizina, Plc-2, Pses3, antifungal activity

1. Introduction

Global losses in agriculture have been estimated of around 35% of annual production due to abiotic and biotic factors (Agrios, 2004). Among the biotic factors, phytopathogenic fungi are the major infectious agents in plants, producing disease and/or substances toxic to human health. Currently, the most effective and widely method to manage plant fungal diseases and to prevent food spoilage with mycotoxins involves chemically-derived fungicide application. The use of fungicides, however, is also associated with limitations such as nonspecific toxicity, emergence of resistance in the fungal population, and limited spectrum of action. In addition, changes in regulatory standards for residue control in food products increasingly requires lower levels of products used to control pathogens, with lower intrinsic toxicity and reduced environmental impact (EFSA, 2010). Importing markets and consumers are increasingly demanding lower levels of pesticides in agricultural products produced following sustainable practices. Tighter regulations have led to some pesticides being banned, and disease management of economically important plants has become more difficult because of the lack of effectiveness of available compounds (Montesinos et al., 2007).

Antimicrobial peptides (AMPs) have been found in virtually all organisms, including prokaryotes and eukaryotes, *e.g.*, mammals, amphibians, insects and plants (Bulet et al., 2004). AMPs are evolutionarily conserved components of the innate immune response and are primary effector molecules (Téllez & Castaño, 2010). The spectrum of activity of various antimicrobial peptides is very broad including antiviral, antifungal and antibacterial activities and, in some cases, anti-tumor and immunomodulatory properties. Most AMPs are cationic, and thus have a positive charge at physiological pH; they are frequently smaller than 100 amino acid residues, and adopt amphipathic structures enabling them to interact with membranes, part of their mechanism of action (Hancock & Sahl, 2006; Marcos et al., 2008).

Different mechanisms have been described for various AMPs allowing for a detailed understanding of their modes of action. Many researchers highlight membrane insertion and association of the peptides into ion-permeable pores leading to the disruption of the cell membrane, but non-lytic mode of action have also been reported (Muñoz & Marcos, 2006). It is known that peptides may interact at three levels: with the outer microbial structures, with the cell membrane and finally with intracellular targets (Marcos & Gandía, 2009). Electrostatic interaction between the plasma membrane and the cationic peptides has been highlighted in all the models so far proposed for the various modes of action of AMPs (Badosa et al., 2009). Marcos and Gandía (2009) suggested that one AMP might combine different killing mechanisms, which potentiate antimicrobial activity and diminishes the risk of developing resistance in the susceptible microbes. At the cellular level, in both microorganisms and higher organisms, qualitative and quantitative differences in the structural composition of cell wall and membrane could explain the effects of the peptides (Table 1).

Table 1. Structural components of cell walls or cell membranes of different classes of organisms (Source: prepared by the Authors based on published information).

	PRINCIPAL CELL WALL COMPONENTS			PRINCIPAL CELL MEMBRANE COMPONENTS		
	Lipids	**Protein**	**Polysaccharides**	**Lipids**	**Protein**	**Net charge**
GRAM-POSITIVE BACTERIA Prokaryote Cell type	Lipoteichoic acid	Mostly lipoproteins and porins	90% peptidoglycan, Teichoic Acid	18% phosphatidylglycerol: anionic Glycerol 65% Phosphatidylethanolamine: Ethanolamine-neutral. Phosphatidylserine Traces:Serine-anionic.	50% variable according to the media	Negative
GRAM-NEGATIVE BACTERIA Prokaryote Cell type	Lipopolysaccharide-LPS,	Mostly lipoproteins and porins.	10% peptidoglycan.	18% phosphatidylglycerol anionic Glycerol 65% Phosphatidylethanolamine: Ethanolamine-neutral. Phosphatidylserine Traces: Serine-anionic.	50% variable according to the media	Negative
FILAMENTOUS FUNGI Eukaryotic Cell type	Absent	20-30% dry weight mannoproteins	50-60% dry weight glucans, β-1, 3, β-1, 6, α-1, 3 D-glucans,galactomannan and mannan10-20% dry weight of chitin	Sphingolipids: Sphingosine + fatty acid: Mannosyl-di-inositol phosphate-ceramide. Sterols: Mostly Ergosterol.	50% variable according to the media	Negative
YEAST Eukaryotic Cell type	Absent	30-50% dry weight mannoproteins	1-2% dry weight glucans, β-1, 3 –β-1, 6-D-glucans galactomannan and mannan 2% dry weight of chitin	Sphingolipids: Sphingosine + fatty acid: Mannosyl-di-inositol phosphate-ceramide. Sterols: Mostly Ergosterol	50% variable according to the media	Negative
PLANT CELL Eukaryotic Cell type	Absent	HRGPs rich hydroxyproline Protein-PRPs Proline-rich proteins GRPs: Rich in glycine proteins and AGPs, arabinogalactan proteins.	Cellulose 20-40% β 1-4 glucose polymers.	Mostly Phytosterols	20-70% varies depending on cell type.	No net charge
ANIMAL CELL Eukaryotic Cell type		Absence of cell wall		7-15% Phosphatidylethanolamine: Ethanolamine-neutral. 10-25% Phosphatidylethanolamine: 10% Phosphatidylserine: Serine-anionic. Sterols: 25% cholesterol.	20-70% varies depending on cell type.	No net charge

Currently, several well characterized natural peptides and derivatives are known to have antimicrobial properties (Hammami et al., 2009; Pelegrini et al., 2011). Among them, Temporins are relatively small AMPs (10 to 14 amino acids) found in skin secretions of amphibians (Ghiselli et al., 2002; Mangoni et al., 2006), that have net positive charge at neutral pH, and an amidated C-terminus. This family of peptides includes more than 40 members showing antimicrobial activity against a broad range of pathogens including Gram positive and negative bacteria and yeasts, but are not toxic to mammalian cells. Temporins adopt amphipathic α-helical conformations in hydrophobic solvents that play an important role in their mechanism of action (Conlon et al., 2004), which is thought to involve disruption of the plasma membrane (Oren et al., 1999). In this study, a 14 amino-acid fragment named Temporizina, was synthesized and rationally designed by the Laboratory of Biochemistry of Proteins and Peptides of the IOC/FIOCRUZ-RJ-Brazil, based on the sequence of three antimicrobial peptides (Souza, 2012) (Table 2).

Plc-2 is a small C-terminal peptide of 11 amino acids derived from Pleurocidin, a linear peptide isolated from the fish *Pleuronectes americanus* (Table 2), also known as flounder or winter flounder (Cole et al., 1997; Souza et al., 2012). This fragment is a cationic peptide with α-helical structure and has antimicrobial activity against Gram positive and negative bacteria and yeast. It is particularly effective against human pathogens that are usually resistant to traditional antibiotic treatments, and it shows a high percentage (> 91%) of lytic activity on *P. aeruginosa,* moderate on *S. aureus* and *E. coli* but no effect on *E. faecalis and M. tuberculosis* growth.

Pses3 is a synthetic fragment of 20 amino acids derived from cationic peptides of sesame kernel (Table 2). Sesame (*Sesamum indicum L.*) is an oleaginous kernel that has been commonly used by several nations as a source of food and medicines. Previous studies have reported the presence of antimicrobial cationic peptides from *S. indicum* kernels of black and white cultivars with activity against human pathogens (Teles et al., 2007).

Table 2. Structural parameters and properties of Plc-2, Temporizina and Pses3

	Peptide		
	Plc-2	Temporizina	Pses3
Sequence	KHVGKAALTHYFLPLWLWLWLWKLKWRQRYYRYVHGYMGPKGYTR		
Molecular weight (g/mol)	1224.4	1942.4	2638
Theorical pI	9.7	10	10.28
Number of residues	11	14	20
Net charge z	(+) 2	(+) 2	(+) 5
Charged residues	2 ,Lys	2 ,Lys	4Arg ,1Lys
Uncharged residues + GLY	1Gly ,1Thr ,2His	0 ,Gly	3Gly ,1Thr 1His ,1Gln
Aromatic residues	1Tyr	1Phe ,4Trp	1Trp 5Tyr
Polar residues + GLY (n / %)	6/55.54	2/14.29	11/55,00
Nonpolar residues (n / %)	5/45.45	12/85.71	9/45.00
Aliphatic index	80	167.14	14.5
Mean relative μHrelb moment CCS	0.29	0.16	0.16
Mean H moment CCS	-1.77	6.21	-2.0
Hydropathicity (GRAVY)	-0.455	-0.90	-1.670
Instability index	33.66	105.01	9.11

Online ExPASy Proteomic server: HYPERLINK http://ca.expasy.org. http://heliquest.ipmc.cnrs.fr/cgi-bin/ComputParams.py and the Antimicrobial Sequences Database and Software: http://www.bbcm.units.it/. HydroMCalc. The relative hydrophobic moment (μHrel for short) of a peptide is its hydrophobic moment relative to that of a perfectly amphipathic peptide. A value of 0.5 indicates that the peptide has about 50% of the maximum possible amphipathicity. The mean hydrophobicity (H for short) calculated by CCS scale is the total hydrophobicity (sum of all residue hydrophobicity indices) divided by the number of residues (with alpha helix projection angle 100º). (Giangaspero et al., 2001).

All peptides used in this work (Temporizina, Plc-2 and Pses3) were rationally designed and produced by the Laboratory of Biochemistry of Proteins and Peptides, FIOCRUZ, (Brazil, see De Simone & Souza, 2002).

The aim of the present study was to evaluate the *in vitro* antifungal activity of three synthetic peptides, derived from natural sources, against seven filamentous fungi with agricultural relevance. The effect of the synthetic peptides on cell permeation of filamentous fungal was evaluated by *in vitro* bioassay and fluorescence microscopy.

2. Materials and Methods

2.1 Synthetic Peptides

Temporizina, Plc-2 and Pses3 were designed and produced by the Laboratory of Biochemistry of Proteins and Peptides, FIOCRUZ, (Brazil). Peptides were synthesized by the solid-phase synthesis method on a PSS-8 (Shimadzu, Kyoto, Japan) Pepsynthesizer according to fluoren-9-methyloxycarbonyl (Fmoc)-polyamide active ester chemistry. Amino acids for peptide synthesis were from Calbiochem-Novabiochem Corp. (Germany).

For the assay, the peptide concentrations ranged from 2-fold serial dilutions. The initial concentrations were 1 $mgml^{-1}$ for each peptides (10X working peptides solutions). As a positive control the fungicide Captan was used at 0.5 $mgml^{-1}$ (Molecular Weight 300.61).

2.2 Phylamentous Fungi

Seven phylamentous fungi were used in the experiments: *Alternaria solani*, *Colletotrichum gloesporioides*, *Fulvia fulvum*, *Fusarium oxisporum*, *Aspergillus niger* and *A. ochraceus* (kindly provided by the Department of Plant Protection, INIA Las Brujas, Uruguay, Table 3) and *Penicillium digitatum* (strain A35, kindly provided by the Department of Plant Pathology, INIA Salto Grande, Uruguay). The fungal isolates were maintained in PDA slants at 4°C till use. Spores of each fungus were obtained from cultures on agar plates (Potato Dextrose Agar (PDA), Oxoid, Hampshire, U.K.) after 7 days at 27°C as described previously (Broekaert et al., 1990). The concentration of the spore suspension was determined using a Thoma-Neubauer cell counting chamber (BOECO, Hamburg, Germany), and suspensions were adjusted to 2 $x10^6$ $sporesml^{-1}$.

Table 3. Phylamentous fungi used in the experiments detailing original plant tissue species and source

Fungal species	Isolate code	Origin	Source
Alterrnaria solani	HBX	Tomato	INIA LB Plant Pathology Lab
Colletotrichum gloesporioides	CC1	Strawberry (crown)	INIA LB Plant Pathology Lab
Fulvia fulvum	STM	Tomato (leaves)	INIA LB Plant Pathology Lab
Fusarium oxysporum f.sp. *lycopersici*	EOA	Tomato (stem)	INIA LB Plant Pathology Lab
Aspergillus niger	RS3	Grape	Dr. Ramos Girona, Lleida
A. ochraceus	RN8	Grape	Dr. Ramos Girona, Lleida
Penicillium digitatum	A35	Orange (fruits)	INIA SG Plant Pathology

2.3 Determination of Antifungal Activity and MIC

The assay for microbial growth was performed on 96-well sterile microtiter plates (Nunc, Roskilde, Denmark) by a quantitative microspectrophotometric method (Broekaert et al., 1990). The final volume per well was 200 μL. Firstly, 20 μL of 10X working peptides solutions were dispensed added in the first column of a microplate. Each row consisted of a serial dilution series for a given peptide. For each well, 180 μL of a spore suspension of

each fungus ($2x10^6$ $sporesml^{-1}$ in fresh PDB, Potato Dextrose Broth, Himedia, India at 70%) were added to 20 μL serial dilution of peptide. In one row 20 μL of sterile distilled water was used instead of peptide, to check for fungal growth without any inhibition. The commercial fungicide Captan, 0.5 $mgml^{-1}$ (Ftalimida, wettable powder), was used to check fungal growth inhibition as a positive control (Satish et al., 2007). The plates were incubated at the appropriate temperature for each organism in an oven, without agitation. Fungal growth was measured after 48h of incubation and OD at 595 nm (Díaz-Dellavalle *et al.* 2011) determined in a Multiskan Spectrum plate spectrophotometer (Thermo Electron Corporation, Finland). For each fungal assay, three replications by treatment and concentration were done. The Minimum Inhibitory Concentration (MIC) was defined as the concentration of peptides (μM) that caused 90% growth inhibition compared with negative controls-the medium without any treatment- after 48 hours. MIC was calculated with the average of the replications.

2.4 Determination of Minimum Fungicide Concentration (MFC)

The *in vitro* fungicidal activity (MFC) was determined as described by Díaz-Dellavalle et al. 2011. For MFC determination we used a subsample collected from the microplates previously incubated for 72 hs for fungal development in media broth amended with the challenged AMP. The subsample was collected from the first wells with no visible growth of the fungi, and was transferred into PDA for a new incubation at 27 ˚C. A positive control (subsample from media broth with water) was also incubated on PDA. For all plates, incubation lasted till fungal growth was observed on control plates. After 72 h of incubation, 20 μL was subcultured from each well that showed no visible growth (growth inhibition greater than 98%), from the last positive well (growth similar to that for the growth control well), and from the growth control (peptide-free medium) onto PDA plates. The plates were incubated at 27°C until growth was seen in the growth control subculture. The minimum fungicidal concentration was regarded as the lowest peptide concentration (μM) that did not yield any fungal growth on the solid medium used. For each fungal isolate, three replications by treatment and concentration were done. MFC was calculated with the average of the replications.

2.5 *Evaluation of the Permeability of Fungal Plasma Membrane*

In order to detect cell lysis, the permeability of the plasma membrane was visualized by fluorescence microscopy using the probe Sytox Green (SG, Invitrogen, USA), which is unable to cross intact biological membranes (Muñoz & Marcos, 2006). To view the fungal structures we simultaneously used the fluorophore Calcofluor White (CW, Sigma-Aldrich, USA) which has high affinity for the chitin in fungal cell walls. Ten μL per well of a stock solution of SG (4 μM in 5% PDB) to a final concentration of 0.2 μM was added to each fungal sample previously exposed to the peptides at different concentrations for at least 48 hours, and then incubated for 5 min in the dark. Five μL of CW 0.1% (w/v) was added to a final concentration of 50 μgml^{-1}. Finally, samples were incubated for 5 min in the dark, washed and mounted on slides in a solution of 20% glycerol. The washing protocol was optimized for each fungus, taking into consideration the macroscopic characteristics of its mycelium, strength, quantity, pigmentation and sporulation rate. In general, mycelia were washed twice, and then harvested by centrifugation after incubation with the probes. Microscopic visualization of the samples was done using a vertical optical photomicroscope, Optiphot-2 (Nikon Corporation, Japan) with an epifluorescence system, a mercury light source (100 W) and filters to visualize SG after excitation at 488 nm and CW after excitation at 355 nm. Photographs were taken with a digital camera, COOLPIX S10 VR, 6.0 megapixels with a 10x zoom and with a microscope adapter (Nikon Corporation, Japan).

3. Results and Discussio

Table 4. MIC and MFC in μM observed in seven filamentous fungal pathogens.

	MIC and MFC μM													
	A. solani		*C. gloesporioides*		*F. fulvum*		*F. oxysporum*		*P. digitatum*		*A. niger*		*A. ochraceus*	
PEPTIDE	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
Plc-2	2,56	Nd	0,64	2,56	5,12	20,5	2,56	5,12	2,56	Nd	1,28	2,56	10,25	>20,5
Pses3	2,37	2,37	1,18	2,37	4,75	>19	1,18	2,37	2.37	Nd	2,37	4,75	>19	>19
Captan	41,6	Nd	5,2	Nd	10,4	Nd	5.2	Nd	1.3	Nd	5.2	Nd	5,.2	Nd

* MIC and MFC were not determined for Temporizina because only values above 90% inhibition were considered for subsequent determination of these parameters. MIC and MIF were calculated with the average of three replications. Nd: Not determined.

As shown in Table 4 and Figures 1, 2 and 3, the growth inhibition of the tested fungi varied depending on the species treated with the different peptides. In general, for the seven fungi evaluated, Pses3 and Pcl-2 were the most active peptides, producing complete growth inhibition. Temporizina, which had a lower overall activity, was significantly active against *Alternaria solani, Penicillium digitatum* and *Fulvia fulvum* with growth inhibition higher than 50% compared with other species of fungi treated (Figure 3). In our conditions, high inhibition values (>90%) were used to estimate MIC and MFC. Interestingly, MIC values obtained for Plc-2 and Pses3 were lower than those for the commercial fungicide Captan when tested on five of the seven fungi species we studied (see Table 4, Figure 1, 2 and 3).

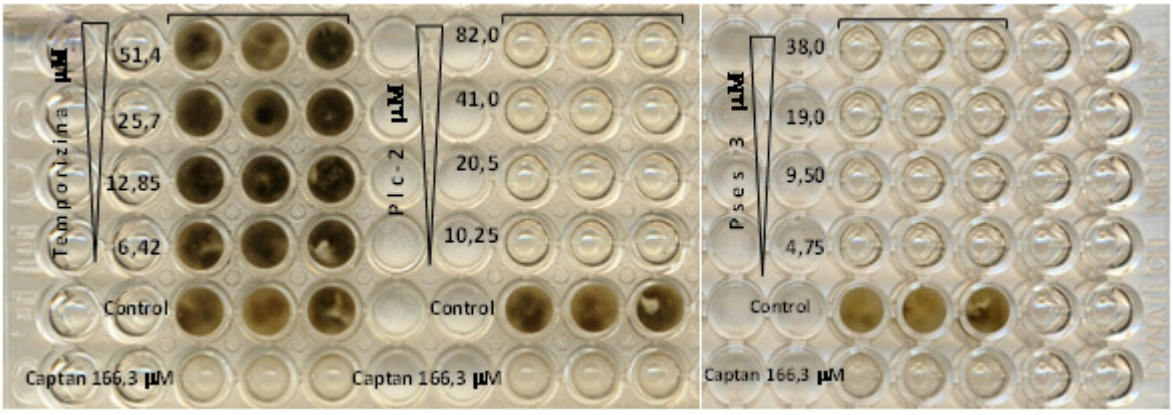

Figure 1. Microplate biosssay showing the effect of the three AMPs on the fungal growth of *Alternaria solani* after incubation for 48hs at 27˚C. For each AMP, four peptide concentrations (in µM) are compared with the synthetic fungicide Captan (positive control) and water (negative control).

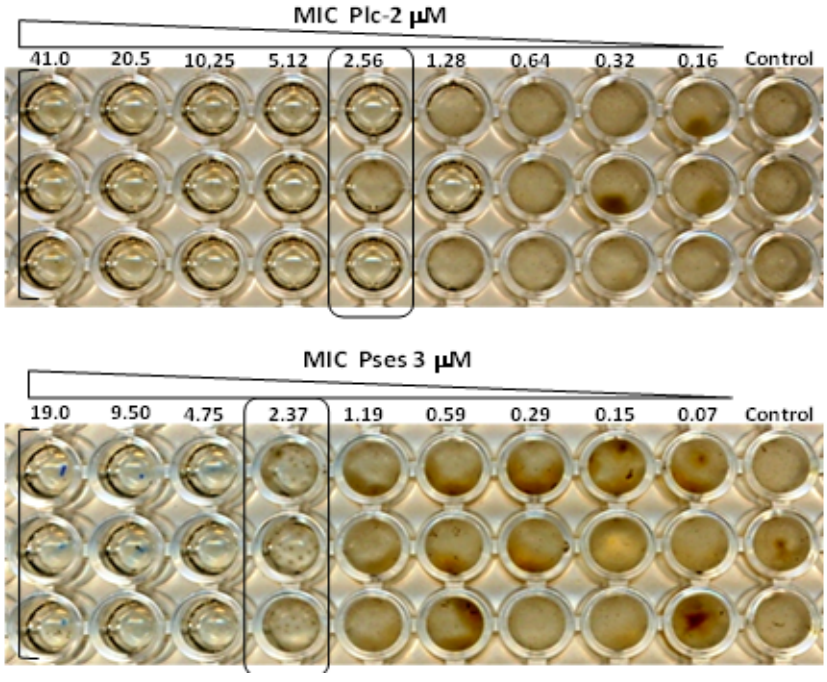

Figure 2. Microplate bioassay for MIC (µM) determination of the AMPs Plc-2 and Pses3 for *Alternaria solani*. The numbers in the figure indicate the AMP concentration in µM, and the Control is the media broth with water. The rectangle indicates the established MIC.

When the susceptibility of the filamentous fungi was compared, the most tolerant were *A. ochraceus* and *F. fulvum*. Among fungi, the values of MFC varied 32-fold for Plc-2 and 16-fold for Pses3. This observation is

especially significant showing that there are compositional differences in the cell wall, membrane constituents and beyond the membrane among fungi that explain a differential response to peptides (Table 1). In this regard, we must remember that these peptides were initially selected for their antibacterial activity. In general, for most of the fungi tested good inhibitory values were obtained with the peptides Plc-2 and Pses3 (Table 4 and Figure 1, 2, 3).

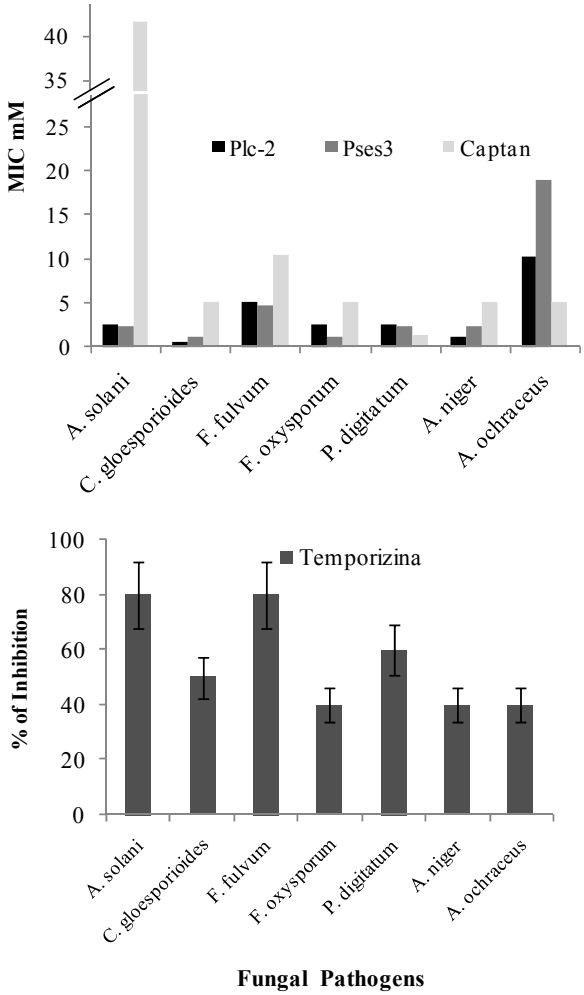

Figure 3. Effects of the treatments on the seven phylamentous fungi. Above: MIC (μM) of Plc-2, Pses3 and Captan observed for seven phytopathogens. Below: Inhibition growth observed on phylamentous fungi caused by Temporizina at 25.7 μM.

An outstanding result from the peptides studied was that the MIC values were always lower than Captan, the positive control (Table 4 and Figure 3), except for *P. digitatum* and *A. ochraceus*. For instance, for *A. solani* and *C. gloesporioides*, the MIC values obtained with Plc-2 were 16.3 and 8.1 times smaller compared to the commercial fungicide. Similarly, for the peptide Pses3 these values were 17.6 and 4.4 times smaller, respectively. However, in the case of *P. digitatum* and *A. ochraceous*, although they completely inhibited the growth of the fungus, their MIC values were larger than that of Captan (Table 4).

Another valuable finding was the observed MFC values, as mentioned above. In effect, fungicidal behaviors were observed for the peptides Plc-2 and Pses3. For these two peptides and for several of the fungi, MFC values were lower than 3 μM (Table 4).

The seven fungi were subjected to concentrations of AMPs close to the MIC values for a given AMP-fungus combination and then examined by microscopy (Figure 4). The potential relationship between fungal growth inhibition and increased cell membrane permeability due to a given AMP was evaluated. Probe SG, in

combination with probe CFW, allows determination of the membrane permeability state as described above.

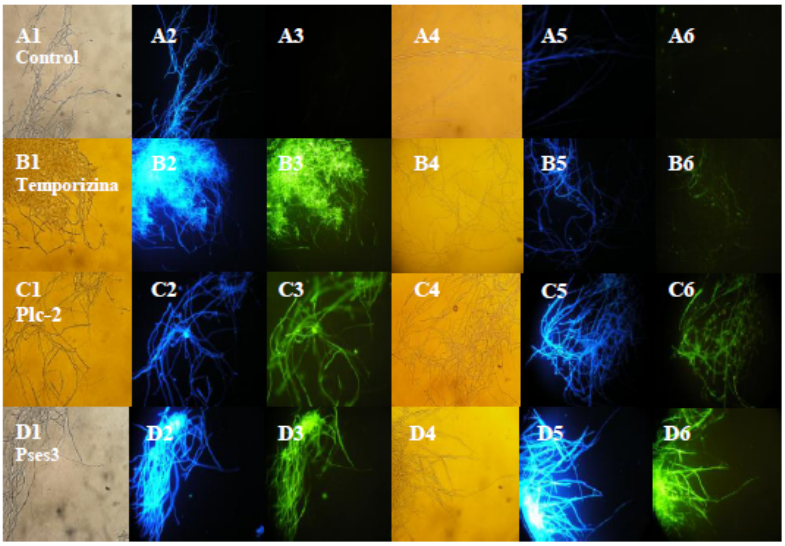

Figure 4. Microscopy visualization after 24 h of the mycelium of *C. gloesporioides* and *P. digitatum* treated with Temporizina, Plc-2, and Pses3. Images of the mycelium from a: Control, b: Temporizina 25.7 µM, c: Plc-2 at MIC concentration, 0.64 µM for *C. gloesporioides* and 2.56 µM for *P. digitatum*, d: Pses3 at MIC concentrations, 1.18 µM for *C. gloesporioides* and 2.37 µM for *P. digitatum.* Panels 1 to 3 and 4 to 6 respectively show the same area viewed in bright field (panels 1 and 4), CFW fluorescence (panels 2 and 5) and SG (Panels 3 and 6).

For Temporizina the values of inhibition varied between 40 and 80% (Figure 3) suggesting that this peptide has different behavior depending on the fungal species. As is known, the components of the external structures of the microorganisms (membrane and cell wall) are highly variable in composition depending on the species, probably affecting the fungicidal activity of the peptides.

When *C. gloesporioides* and *P. digitatum* were treated with Temporizina at concentrations that produce percentages of inhibition near 50% and 60% respectively (Figure 3), strong emission of SG fluorescence was amazingly observed, for these two fungi only. In fact, this might indicate that exposure to Temporizina permeates the fungal cell even at partially inhibitory concentrations (Figure 4). While the higher fungi were all imperfect and Deuteromycetes, the behavior of peptides was differential with different species, suggesting that the interaction depends on factors such as the composition of the wall or cell membrane (chitin content, sterols, glycoproteins, lipids, etc, Table 1). Plc-2 and Pses3 caused the permeation of the membrane of all fungal species treated at any concentration near to their MIC values (see Table 4 and Figure 4).

As noted above, many factors need to be investigated to determine the specific interactions between AMP and microorganism. It is widely accepted that the mode of action of Temporins and many of the cationic AMPs in bacteria is permeabilization of the plasma membrane with subsequent cell lysis (Bowman and Free, 2006). Nevertheless, other studies have indicated that cell permeation may not be part of a general mechanism leading to the lethal action of the peptide (Mangoni, 2006), and this is in accordance with our results. Temporizina shows high values of inhibitory activity in *A. solani* and *F. fulvum* without evidence of permeation as in *C. gloesporioides* and *P. digitatum* (data not shown).

Some authors reported alteration of the *E. coli* membrane at concentrations of Temporins below that of growth inhibition (Mangoni *et al.*, 2004). This finding is consistent with our results for the species *C. gloesporioides* and *P. digitatum* where lipid composition seems to be a key factor for the activity (Turk et al., 1987, Wassef et al., 1985). Fungal species treated with Plc-2 and Pses3 peptides resulted in 100% growth inhibition and low values of MIC and caused membrane disruption and high levels of fluorescence emission in all species using the SG probe (Table 4, Figure 4). This study using imperfect fungi and Deuteromycetes may complement previous efforts to determine what microorganisms are affected by Plc-2 as well as what types of mechanisms are likely to be involved. Many reviews indicate (Cole et al., 1997, Jung et al., 2007; Thevissen et al., 1999) that Pleurocidina,

from which Plc-2 is derived, exerts antimicrobial activity mainly through intracellular targets in microorganisms such as *Saccharomyces cerevisiae*, *Candida albicans*, *Malassezia furfur*, *Trichosporon beigelli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*, without cellular permeation. In our study, for all the filamentous fungal species tested, inhibition was accompanied by cell permeabilization, leading to think that the mode of action in fungal species is specific and different from bacteria.

In Table 2, the structural parameters and properties data of the three antimicrobial peptides used in this study are shown. The peptides assayed in this study have positive net charge, with different hydrophobicity. Previous reports established a relationship between the cationic nature of peptides at physiological pH and their antimicrobial activity (Brogden, 2005; Jenssen et al., 2006). The cationic nature of many AMPs involves an electrostatic attraction to a cell surrounded by negative charge, as in the case of lipopolysaccharide (LPS) of Gram negative bacteria, or glycoproteins and glycosphingolipids of the fungal wall. In fact, this is a plausible reason for specificity against microorganisms and the absence of toxicity against animal and plant cells (Zasloff, 2002; Alves et al., 2010). Peptide hydrophobicity is another important parameter related to antimicrobial activity, and in our case it varied greatly among the peptides studied, being particularly high in the case of Plc-2.

The relationship between structure and function of each peptide must, in turn, be closely related to the characteristics of the microorganism to be controlled. Through biotechnological approaches, the AMPs are one of the most promising alternatives to chemical control. In fact, after the isolation of natural AMPs, the application of combinatorial chemistry allows synthetic peptides to be designed with more effective antimicrobial activities and with advantageous properties. These multifunctional molecules are likely to have a great future as new biotech molecular biocides.

In conclusion, this work compares peptides previously known for their antibacterial activity, for antifungal activity against seven phylamentous fungi isolated from plant tissue. Peptides Pses3 and Plc-2 showed remarkable antifungal activity in all tested fungi. Pses3, derived from sesame seeds, is a novel peptide, so far not reported in databases, with promising characteristics for use in the control of pathogens in the food industry.

Acknowledgements

This work received financial assistance from CNPq, DICyT-MEC, CAPES, FAPERJ-Fo SGS. We thank the peptide synthesis platform of the Center for Technological Development in Health (CDTS-PDTIS) of FIOCRUZ for the use of their facilities. We are grateful to our co-workers who supplied fungal isolates and provided useful suggestions and information for this study. Kind thanks to: Nora Altier (Department of Plant Protection, INIA Las Brujas), Elena Pérez (Department of Plant Pathology, INIA Salto Grande) and Fernando Rivas (National Citrus Production, INIA Salto Grande). We are grateful to Dr. José Francisco Marcos for his critical review of the manuscript.

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