Comparison of the activity of antifungal hexapeptides and the fungicides thiabendazole and imazalil against postharvest fungal pathogens.

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Abstract

In this study, we evaluated the activity of short antimicrobial peptides against different fungal isolates that cause postharvest decay of fresh fruits. The previously identified hexapeptides PAF19, PAF26 and LfcinB$_{4-9}$ inhibited the *in vitro* growth of isolates from *Penicillium digitatum* and *P. italicum*, and from *Alternaria* and *Geotrichum* genera, being no active against *Rhizopus, Mucor* and *Aspergillus*. The results extend our previous observations on the specific and distinct activity profiles of PAFs. In addition, peptide activities were compared with that of two fungicides used for citrus fruit preservation, thiabendazol (TBZ) and imazalil (IMZ). We observed a lack of correlation between peptide and fungicide sensitivity among different species. Importantly, *P*. *digitatum* and *P*. *italicum* isolates resistant to fungicides were susceptible to peptides, and our data suggest that common multiple drug resistance mechanisms are not active against this class of peptides. The *in vitro* peptide inhibition was correlated with a retard of the decay caused by *Penicillium* on citrus fruits, and this effect was comparable for both fungicide-resistant and -sensitive isolates. Comparison of PAF26 and TBZ *in vitro* MIC values and their *in vivo* effect on citrus decay indicated that PAF26 performed *in vivo* better than TBZ.

Keywords: antimicrobial peptides, fungicides, *Penicillium*, Citrus fruits, postharvest.

1. Introduction

There is an obvious need to search for alternative strategies/compounds to control fungal plant diseases (Knight et al., 1997). A paradigm is postharvest storage, wherein losses are controlled, mainly, through massive application of toxic chemical fungicides (Barkai-Golan, 2001). Antimicrobial peptides have potential as food-grade additives (Delves, 1990; Schillinger et al., 1996), and it has been proposed their use to fight phytopathogens in agriculture (Rao, 1995; van der Biezen, 2001) and postharvest conservation (López-García et al., 2000). The design of new synthetic non-natural peptides could circumvent some of the problems associated with certain antimicrobial peptides (for instance, toxicity or stability). A chimerical gene coding for a peptide hybrid of cecropin and melittin has been successfully expressed *in planta* (Osusky et al., 2000), and another such hybrid with known antimicrobial properties against fungal pathogens (Cavallarin et al., 1998; Ali and Reddy, 2000) was produced in *Sacharomyces*, which was applied to *Colletotrichum*-inoculated tomato fruits (Jones and Prusky, 2002). In previous works, we used combinatorial chemistry to design a group of hexapeptides with specific antifungal activity against selected phytopathogens, so-called PAFs (Peptide and AntiFungal). Two of these peptides were further characterized and are PAF19 (López-García et al., 2000) and PAF26 (López-García et al., 2002), with similar sequences (Table 1), albeit distinct potency.

The two major postharvest diseases of citrus are green and blue rots, caused by *Penicillium digitatum* Sacc. and *P. italicum* Wehmer, respectively (Eckert and Ogawa, 1985; Barkai-Golan, 2001). *Geotrichum candidum* and *Alternaria* spp.

have lower incidence but might become problematic whenever *Penicillium* rots are successfully controlled. Fungicides belonging to the benzimidazole [thiabendazole (TBZ) and benomyl] and imidazol [imazalil (IMZ)] groups are commercially applied to control postharvest citrus decay, although they are ineffective (or less effective) against *Geotrichum* spp., *Rhizopus* spp. or *Alternaria* spp. Also, resistant strains have been described (Eckert, 1988; Bus, 1992), mainly because of intensive fungicide use. TBZ and IMZ are systemic fungicides that act on specific targets in such a way that mutations in the corresponding genes might develop resistance. TBZ and benomyl act avoiding tubulin polymerization and inhibit mitosis. IMZ is an inhibitor of ergosterol biosynthesis. Many fungi develop resistance to multiple unrelated chemicals, the so-called multidrug resistance (MDR), and ATP-binding cassette (ABC) membrane transporters have been shown to play a role in MDR and/or pathogenesis (Nakaune et al., 1998; Urban et al., 1999; Schoonbeek et al., 2001).

PAFs have promising activities against *P*. *digitatum* and *P*. *italicum*. In order to assess their potential use in postharvest treatments, it is necessary to evaluate PAF activities against broad collections of isolates and, importantly, to compare them with that of commercial fungicides. The present study was conducted with this double objective, by assaying PAF19, PAF26, TBZ and IMZ against isolates belonging to *Penicillium, Geotrichum, Rhizopus*, *Mucor*, *Alternaria,* and *Aspergillus* genera. Also, we had previously noted that the antibacterial core of lactoferricin, the L-amino acid hexapeptide LfcinB$_{4\text{-}9}$ (Table 1), has strong sequence similarities with PAF19 (López-García et al., 2000); this

peptide was included to test whether this resemblances are reflected in similar antifungal properties.

2. Materials and methods

2.1. Fungal isolates.

In this study, we used a collection of fungal isolates, all of them related with citrus postharvest pathogenesis (Table 2). Fungi were cultured on potato dextrose agar (PDA) (Difco, Detroit, USA) plates at 24ºC. Conidia were collected by adding sterile water to the surface of the mycelium and gently scrubbing with a glass rod, or by scraping them from the agar with a sterile spatula and transferring to sterile water. Conidia were then filtered, and titrated with a hemacytometer.

2.2. Peptides and fungicides.

Peptides (Table 1) were synthesized in-house by solid-phase methods using N-(9-fluorenyl) methoxycarbonyl (Fmoc) chemistry (Fields and Noble, 1990) and purified by preparative reverse phase high-pressure liquid chromatography (RP-HPLC), as previously described in detail (López-García et al., 2000). Their identities were confirmed by MALDI-TOF (matrix-assisted laser desorption/ionization time-of-flight) mass spectrometry. Stock solutions of each peptide were prepared at 1 mM in 5 mM 3-(N-morpholino)-propanesulfonic acid (MOPS) pH 7 buffer. Peptide concentrations were determined by measuring the absorbance at 280 nm and re-checked before each experiment.

Fungicides used were thiabendazole (TBZ) (Purity 99.5%, Sigma, St. Louis, USA) and imazalil (IMZ) (Purity 99.9%, Riedel-de-Haën, Seelze, Germany). Stock solutions of each fungicide were prepared at 1 mg/ml in 5 % ethanol.

2.3. In vitro antimicrobial activity assays.

Fungi were grown at 24ºC on sterile 96-well microtiter plates (Nunc, Roskilde, Denmark) in a final volume of 200 μl and growth was determined by measuring the absorbance at 492 nm with a Titertek Multiskan PLUS reader (Labsystems, Helsinki, Finland) as previously described (López-García et al., 2000). In the assay mixture, 130 μl of potato dextrose broth (PDB) (Difco, Detroit, USA) containing 0.003% (wt/vol) chloramphenicol was combined with 50 μl of PDB containing 10^5 conidia/ml of each fungi and 20 μl of each peptide or fungicide, added from 10x stock solutions. Final peptide molar concentrations used were 10, 20, 40, 60, and 80 μM. Final fungicide concentrations for experiments shown in Table 2 were 1, 5, 10, 50 and 100 μg/ml. Fungicide concentrations were subsequently transformed to molar concentration for data presentation (molecular weights 201.20 for TBZ and 297.18 for IMZ). In all the experiments, each treatment was carried out as triplicate samples (three wells in the plate) and the mean and standard deviation (SD) (after background subtractions) were calculated for each. The experiments were replicated at least twice for each fungal strain. The MIC was defined as the lowest compound concentration that showed no growth at the end of the experiment (usually after 3 days of incubation). The IC_{50} was calculated as described previously (López-García et al., 2000). For this purpose, the final concentrations in the case of fungicides were 10, 50, 100, 150, 200, 250, 300, 400, 500, 750 and 1000 ng/ml.

2.4. Fruit decay tests.

Experiments were carried out on freshly harvested orange fruits (*Citrus sinensis* L. Osbeck cv. Navelina). Fruits were submerged for 5 min in a 5% commercial bleach solution (equivalent to 0.25% free chlorine), washed subsequently with tap water and allowed to dry. Fruits were wounded by making punctures (approximately 5 mm in depth) with a nail at four sites around the equator. Fungal strains used in these experiments were *P. digitatum* PHI-26 and PHI-41, and *P. italicum* PHI-1 and PHI-52. Inoculums contained 10^4 conidia/ml and antifungal compounds (either peptides or TBZ, at desired final concentrations), and 10 μl of the inoculum were applied onto each wound. For each treatment, three replicas (five fruits per replica, four wounds per fruit) were prepared in each experiment. Fruits were maintained at 20ºC and 90% RH. Symptoms were scored at different days post inoculation (dpi) as the number of infected wounds in each replica. The percentage of infected wounds and mean values ± SD for each treatment were subsequently calculated. Statistical analyses were carried out with the software package StatGraphics Plus 4.0 (Manugistics Inc., Rockville, USA). The F test was applied to test if difference between the treatment means was significant, and the Turkey's honestly significant difference (HSD) procedure was used for mean separation.

3. Results

3.1. Inhibition of in vitro growth of natural fungal isolates.

Synthetic hexapeptides previously identified as antimicrobials were assayed against a collection of fungal isolates (Table 2). These hexapeptides were

PAF19 and PAF26 identified in our group by a combinatorial chemistry approach (López-García et al., 2000; López-García et al., 2002), and the active center of lactoferricin B ($LfcinB_{4-9}$) (Tomita et al., 1994), a peptide of natural origin whose sequence has resemblances of PAF19 (López-García et al., 2000) (Table 1). Our results confirmed that PAFs are active against distinct isolates of the most important fungi causing postharvest decay in citrus fruits, *Penicillium digitatum* and *P*. *italicum*, and demonstrated also the activity of $LfcinB_{4-9}$. *O*ther *Penicillium* spp. were found that were less affected or even insensitive to PAFs (i.e. PHI-8 and PHI-65).

The spectra of action of PAFs and the lactoferricin peptide were equivalent. MIC values of $LfcinB_{4-9}$ and PAF19 were similar among the different strains tested, while PAF26 showed consistently stronger activity (i.e., lower MIC values). $LfcinB_{4-9}$ cationic charge and amino acid sequence are more related to PAF19 than to PAF26, which could explain its antifungal properties. It is worth noting, in addition, that $LfcinB_{4-9}$ used in this work was synthesized with the natural L-enantiomers of amino acids. It has been reported that the PAF19 sequences synthesized with either D- or L-amino acids share comparable activities, albeit the former is more resistant to proteolytic degradation (López-García et al., 2000).

The data obtained with the hexapeptides were compared with the fungicides TBZ and IMZ (Table 2). Some natural isolates of *Penicillium* with reduced sensitivity to TBZ and IMZ were detected in our collection. It has been reported that IMZ^R isolates of *P*. *digitatum* were frequent in California packing-houses, while resistant *P*. *italicum* were rare (Holmes and Eckert, 1999). Our study, although with a much lower number of isolates, would confirm this previous

study. Importantly, we did not find a correlation between sensitivity to PAFs and sensitivity to either TBZ or IMZ. For instance, the fungicide-resistant *P. digitatum* and *P. italicum* isolates were sensitive to PAFs (i.e. PHI-35, PHI-41 and PHI-52). The opposite sensitivity behaviour was also observed; the PHI-8, a PAF^R *Penicillium* sp., was as sensitive to fungicides as *P. digitatum or P. italicum*.

In our assays, we also included some isolates of other fruit pathogens (mainly of citrus) belonging to the genera *Geotrichum*, *Rhizopus*, *Mucor, Aspergillus* and *Alternaria*. Both IMZ and TBZ are known to be ineffective against sour rot caused by *Geotrichum candidum* (Eckert and Ogawa, 1985), and our data confirmed absence of *in vitro* inhibition. Two isolates of *Geotrichum* sp. were inhibited by PAF26 at the highest concentration tested (80 μM). Benzimidazol fungicides are not active against *Alternaria*, and although IMZ has been reported to suppress decay caused by this fungus (Spalding, 1980; Prusky and Ben-Arie, 1981) it does not control it effectively on citrus probably due to its low sensitivity to this fungicide (Table 2). Interestingly, *Alternaria* sp. show a sensitivity to PAFs very similar to *P. italicum,* as noted in comparisons of the MIC data of the peptides against the isolates PHI-2, PHI-4 and PHI-6, with the data against *Alternaria* PHI-44, for instance. We also confirmed, with natural isolates different to the collection strains reported previously (López-García et al., 2000), that *Rhizopus* spp. and *Aspergillus* spp. were not sensitive to PAFs. Thus, our results demonstrate activity of PAFs against some fungi not effectively controlled by some of the current chemical fungicides, and also against isolates of *P. digitatum* and *P. italicum* that have developed resistance to fungicides. Indirectly, data presented also confirm and

extend previous observations on the specific and distinct activity profiles of PAFs. As an internal control of our assays, the inactive P20 peptide (López-García et al., 2000) (Table 1) showed no activity against any of the tested fungi (data not shown).

On the other hand, we observed no synergy (i.e. additive interactions), between fungicides (TBZ or IMZ) and PAF26 on the growth inhibition of *P. digitatum* PHI-26 in experiments designed following the Abbot approach (Abbot, 1925), as described recently (Gisi, 1996) (data not shown).

Four classes of fungi were identified among all the *Penicillium* tested (representative of the different susceptibilities to PAFs and fungicides), and further characterization was carried out by calculating the IC_{50} values of the compounds against them (Table 3): (i) isolates sensitive to both PAFs and fungicides (such as *P*. *digitatum* PHI-26 and *P*. *italicum* PHI-1), (ii) isolates sensitive to PAFs and resistant to fungicides (PHI-41 and PHI-52), (iii) isolates resistant to PAFs and with lower sensitivity to fungicides (the unidentified *Penicillium* PHI-8), and (iv) an isolate resistant to PAFs and TBZ (PHI-65).

3.2. Effect of PAFs on infection of citrus fruits by P. digitatum.

Since PAFs are able to inhibit the *in vitro* growth of fungi which are unaffected by fungicides, we designed experiments to compare the effect of hexapeptides on the infection caused by isolates of *P*. *digitatum* and *P*. *italicum* that were either fungicide sensitive (PHI-1 and PHI-26) or resistant (PHI-41 and PHI-52). A representative experiment (Fig. 1) shows that the *in vitro* growth inhibition was correlated with an effect on the *in vivo* infection, confirming also that PAF26 has a more potent antifungal activity than PAF19. It is remarkable

that both fungicide resistant and sensitive isolates were affected by PAFs to the same extent.

We also compared the *in vivo* antifungal activities of PAFs and fungicides (as example, TBZ) under comparable laboratory inoculations (Fig. 2). Noteworthy, a concentration 3 times the MIC of TBZ was not enough to affect the *in vivo* infection, while a concentration (50 μM) of PAF26 that is 2-3 times higher than its MIC retards the infection of *P. digitatum* PHI-26. It was necessary roughly 150 times the MIC of TBZ to control the infection to the same extent as PAF26 at 50 μM.

4. Discussion

The postharvest application of short synthetic antimicrobial peptides is an attractive alternative to fungicides (López-García et al., 2000; Jones and Prusky, 2002; López-García et al., 2002). In this context, it is important (i) the assessment of peptide antifungal properties against collections of field isolates from natural populations, and (ii) the comparison of peptide activity with that of commercial fungicides.

We describe the comparison of the activity of PAF19 and PAF26 with that of the hexapeptide LfcinB$_{4-9}$ (Tomita et al., 1994), and two fungicides, TBZ and IMZ, against a collection of postharvest fungal isolates, as representative of the populations found in citrus packinghouses from our industrial environment. An emphasis was made to include *P. digitatum* and *P. italicum* isolates that were not only sensitive but also resistant to these common fungicides. Isolate origins included rotten fruits (oranges, mandarins or lemons, either fungicide-treated or non-treated), the surfaces from healthy non-treated fruits, contact plates from

surfaces of packing-house equipment, or water from drenchers containing high doses of fungicides (Table 2).

We have found a new application for the antibacterial core of bovine lactoferricin in the control of postharvest fungal infections. Lactoferrins are iron-chelating proteins present in the milk of mammals, and Lactoferricin B is a 25-residue antibacterial peptide isolated after cleavage of the bovine lactoferrin (Bellamy et al., 1992). Its residues 4-9 ($LfcinB_{4-9}$) have been characterized as its active core and when the C-terminus is amidated have been show to be as potent as the longer peptide (Tomita et al., 1994). In our study, this peptide was synthesized with L-estereoisomers of amino acids (and also with both termini protected, for a better comparison with PAF19 and PAF26), and was found to have potency and spectrum of action similar to PAF19 (Table 2).

An important issue that must be addressed as related to the use of this class of peptides is the evaluation of their potential toxicity or allergenicity effects. The absence of toxicity of PAF19 and PAF26 to bacteria and yeast (López-García et al., 2000; López-García et al., 2002), and their sequence (Table 1) and activity (Table 2) similarities with the antimicrobial center of a natural protein found in mammals look very promising in this regard. Moreover, human lactoferrin has been successfully expressed in potato plants and antibacterial activity against human pathogens was recovered from tubers (Chong and Langridge, 2000).

Our report also confirms that *P. italicum* is less susceptible *in vitro* to PAFs than *P. digitatum* (Tables 2 and 3). It remains to be determined whether this behavior indicates that *P. italicum* has overall less susceptibility to antimicrobial peptides/proteins, or is rather a consequence of the fact that the initial combinatorial design of PAFs was targeted to *P*. *digitatum*. A paradox, however,

is that PAF protection against blue mold caused by *P*. *italicum* seems better than against green mold (Fig. 1). This is probably related with the lower virulence *in vivo* of *P*. *italicum* (i.e., lower progression of infection) as compared with *P*. *digitatum*, when equal number of conidia are inoculated under the same conditions (Fig. 1, and unpublished observations). Therefore, we relate a lower virulence with a higher apparent PAF sensitivity *in vivo.* We observed the same trend in *in vitro* growth experiments in 5% PDB (instead of 100% PDB), in which a much lower growth rate of the fungi lowered the apparent MIC and IC_{50} values of PAFs (data not shown). This observation points to the need of standardization whenever peptide activities are compared among different laboratories.

Our data demonstrate a lack of correlation between peptide and fungicide sensitivity. A relevant finding is that PAFs are capable of inhibiting fungi that are unaffected by frequently used fungicides (as *Alternaria* sp), or that have developed fungicide resistance, likely because of the high selection pressure encountered in packinghouses. Several of the *P. digitatum* and *P. italicum* isolates assayed were resistant to fungicides and sensitive to PAF26 *in vitro* (Table 2). These data are enforced by the very recent demonstration of *in vitro* activity of other short peptides against TBZ-resistant strains of another unrelated fungus, *Fusarium sambucinum* (González et al., 2002). Moreover, the infection of our fungicide resistant isolates was delayed by co-inoculation with the peptide, to the same extent as that of fungicide sensitive ones (Fig. 1). These observations demonstrate that the underlying mechanisms that confer PAF/fungicide sensitivity are independent. Also, double-resistant isolates to the unrelated TBZ and IMZ (i.e. *P. digitatum* PHI-35, PHI-41 and PHI-66) have

likely developed a MDR-like type of resistance, based on the extrusion of toxic compounds mediated by ABC transporters, as already showed in *P*. *digitatum* (Nakaune et al., 1998). Since these isolates are sensitive to PAFs, our data would indicate that such MDR mechanism(s) are not effective against PAFs. We have determined experimentally that a primary consequence of peptide action is the disruption of cell membrane (manuscripts in preparation), as was previously reported for other antimicrobial peptides (Reed et al., 1997; Thevissen et al., 1999; Oren et al., 1999; González et al., 2002).

Our controlled inoculation experiments could seem discouraging at a first sight, since co-inoculation of fungi with PAFs at concentrations close to their *in vitro* MIC only achieved a delay in disease progression (Fig. 2), as we already had reported and attributed it to germinating mycelia growing out of the inoculation site, where peptide is absent. Two important facts must be considered, however. The inoculum dose used was very aggressive and rendered nearly 100% of infection at 4-6 dpi. Also, in the comparison with fungicides it must be considered that they are commercially applied onto the entire fruit surface and at concentrations that are orders of magnitude higher than their *in vitro* MIC value, in order to attain effective protection. In the experiments shown here, the peptides were only applied at the inoculation site. Parallel experiments with TBZ (Fig. 2) demonstrated that under equal conditions PAF26 and TBZ performed similarly when concentrations are expressed on a weight per volume basis, while the result favours PAF26 when the effective molar concentration and its relation to the MIC value are considered.

We found *Penicillium* isolates unaffected by PAF26 (i.e., PHI-8 and PHI-65) and, strikingly, they were not identified as *P. digitatum* or *P. italicum*, the two

pathogens of citrus. In fact, PHI-8 is unable to infect orange fruits (unpublished results). Further experiments will be set to explain this observation. Overall, our results provide evidence to support the investigation and development of the use of PAFs as an alternative to fungicides for controlling postharvest diseases and maintaining food quality.

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Figure Captions

Fig. 1. Effect of hexapeptides on the infection of citrus fruits by *P. digitatum* PHI-26 and PHI-41, and *P. italicum* PHI-1 and PHI-52. Orange fruits were inoculated with 10^4 conidia/ml either alone (hatched bars), or in the presence of 50 μM of hexapeptide PAF19 (white bars) or hexapeptide PAF26 (black bars). Bars show the mean values of the percentage of infected wounds ± SD at 4 days post inoculation. There was a statistically significant difference between the means of the three treatments for the fungi PHI-41, PHI-1 and PHI52, at the 95.0% confidence (F test), and bars labelled with the same letter do not differ at the 95.0% confidence (Turkey's HSD procedure). In the case of PHI-26, the mean of the PAF26 treatment was different from the control at the 95.0% confidence (t-student test).

Fig. 2. Effect of hexapeptide PAF26 and fungicide TBZ on the fungal infection of citrus fruits. Orange fruits were inoculated with 10^4 conidia/ml of *P. digitatum* PHI-26 alone (hatched bars), or in the presence of PAF26 at 50 μM (black bars), or TBZ at 5 μM (white bars) and 250 μM (grey bars). Results are shown as the mean of the percentage of infected wounds ± SD for each treatment and day post inoculation. There was a statistically significant difference between the means of the four treatments for each dpi, at the 95.0% confidence (F test), and bars labelled with the same letter do not differ at the 95.0% confidence (Turkey's HSD procedure).

Table 1

Amino acid sequences of peptides [a]

PAF19	Ac-	r	k	t	w	f	w	$-NH_2$
PAF26	Ac-	r	k	k	w	f	w	$-NH_2$
P20	Ac-	r	k	t	p	f	w	$-NH_2$
$LfcinB_{4-9}$	Ac-	R	R	W	Q	W	R	$-NH_2$

[a] The L-amino acids are shown in capital letters, and the D-amino acids are shown in lower case. The N-terminus of the peptides was acetylated and the C-terminus was amidated.

Table 2

Minimum inhibitory concentration (MIC) of peptides and fungicides against fungal isolates from different origins

Identification	Isolate	Origin	Year	MIC (μM)				
				PAF19	PAF26	LfcinB$_{4-9}$	TBZ	IMZ
P. digitatum	PHI-26	Rotten orange	1999	60	20	60	1.5	0.8
P. digitatum	PHI-35	Rotten mandarin (TBZ-treated)	2000	80	40		>497 [a]	33.6
P. digitatum	PHI-41	Water from drencher with IMZ	1999	80	40	80	NI	33.6
P. digitatum	PHI-42d	Water from drencher with IMZ	1999	60	20		5	3.4
P. digitatum	PHI-43	Fruit surface	1999	60	20		5	
P. digitatum	PHI-66	Rotten lemon	2000	>80 [a]	40		NI	33.6
P. italicum	PHI-1	Rotten mandarin	1997	60	40	60	1.5	1
P. italicum	PHI-2	Rotten orange	1998	>80	40		5	3.4
P. italicum	PHI-4	Rotten orange	1998	>80	40		5	3.4
P. italicum	PHI-6	Rotten orange	1998	>80	40		5	3.4
P. italicum	PHI-36	Rotten mandarin (TBZ-treated)	2000	80	40		25	
P. italicum	PHI-42i	Water from drencher with IMZ	1999	80	60		NI	3.4
P. italicum	PHI-52	Rotten orange	2000	>80	60	80	NI	3.4
P. italicum	PHI-53	Rotten orange	2000	>80	60			
P. expansum	PHI-65	Rotten lemon	2000	NI [b]	NI	NI	NI	1.3
Penicillium sp.	PHI-8	Contact plate from packing-house	1999	NI	NI	NI	2.5	1.7
Rhizopus sp.	PHI-39	Orange surface	1999	NI	NI		NI	
Alternaria sp.	PHI-44	Orange surface	1999	>80	40		NI	168
Alternaria sp.	PHI-48	Rotten orange	1999	>80	60	>80	>497	168
Alternaria sp.	PHI-49	Rotten orange	2000	>80	60	>80	NI	168
Geotrichum sp.	PHI-55	Rotten orange	2000	>80	80	NI	NI	336
Geotrichum sp.	PHI-56	Rotten orange	2000	>80	80	NI	NI	336
Mucor sp.	PHI-50	Rotten orange	2000	NI	>80	NI	NI	NI
A. niger	PHI-67	Rotten grape	2001	NI	NI	NI	>497	>336

[a] The symbol '>' denotes some (but not complete) inhibition of growth at the maximum concentration used of peptide or fungicide (80 μM peptide, 497 μM TBZ, or 336 μM IMZ).

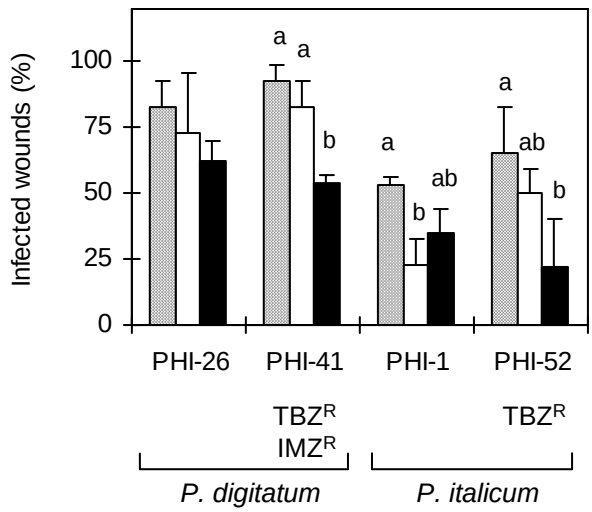
[b] Not inhibitory (i. e., no significant effect observed on growth) at the maximum concentration used of peptide or fungicide.

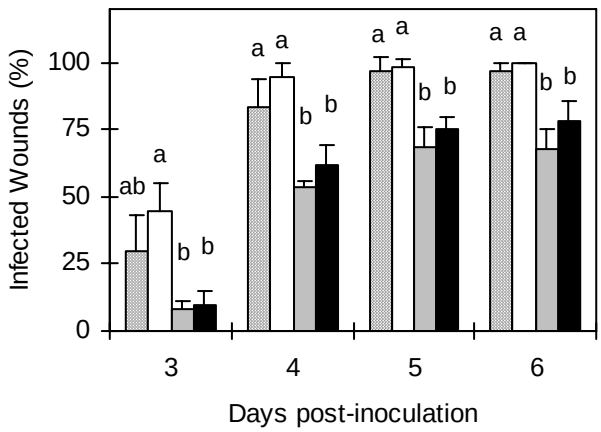
Table 3

IC_{50} of the peptides PAF19 y PAF26 and the commercial fungicides TBZ and IMZ against selected isolates of the genus *Penicillium*.

		IC_{50} (μM)			
		PAF19	PAF26	TBZ	IMZ
PHI-26	*P. digitatum*	38 ± 5	12 ± 2	0.88 ± 0.10	0.49 ± 0.03
PHI-41	*P. digitatum*	39 ± 3	12 ± 2	NI	17.57 ± 1.21
PHI-1	*P. italicum*	40 ± 5	20 ± 5	0.87 ± 0.10	0.57 ± 0.01
PHI-52	*P. italicum*	58 ± 4	31 ± 2	NI	0.66 ± 0.06
PHI-8	*Penicillium* sp.	NI [a]	NI	1.58 ± 0.02	1.25 ± 0.29
PHI-65	*P. expansum*	NI	NI	NI	0.65 ± 0.01

[a] Not inhibitory (i. e., no significant effect observed on growth) at 100 μM peptide or 336.5 μM TBZ.


Infected wounds (%)
100
75
50
25
0
a
a
b
a
b
ab
a
ab
b
PHI-26
PHI-41
PHI-1
PHI-52
TBZR
IMZR
TBZR
P. digitatum
P. italicum


Infected Wounds (%)
100
75
50
25
0
ab
a
b
b
a
a
b
b
a
a
b
b
a
a
b
b
3
4
5
6
Days post-inoculation