Therapeutic potential of antifungal plant and insect defensins

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To defend themselves against invading fungal pathogens, plants and insects largely depend on the production of a wide array of antifungal molecules, including antimicrobial peptides such as defensins. Interestingly, plant and insect defensins display antimicrobial activity not only against plant and insect pathogens but also against human fungal pathogens, including *Candida* spp. and *Aspergillus* spp. This review focuses on these defensins as novel leads for antifungal therapeutics. Their mode of action, involving interaction with fungus-specific sphingolipids, and heterologous expression, required for cost-effective production, are major assets for development of plant and insect defensins as antifungal leads. Studies evaluating their *in vivo* antifungal efficacy demonstrate their therapeutic potential.

During the past 20 years, the incidence of invasive fungal infections (mycoses) in humans has increased considerably, especially because of increased occurrence of immunocompromised patients and increased use of catheters and implants. *Candida* bloodstream infections have steadily increased since the 1980s and account for 8–15% of all bloodstream infections [1,2]. A number of surveys have revealed that in the 1980s more than 75% of *Candida* infections were caused by *C. albicans* [3]; however, non-*C. albicans* species such as *C. glabrata* are being increasingly isolated as causal agents [4]. Filamentous fungi belonging to the *Aspergillus* genus, especially *A. fumigatus* and *A. flavus* are other important causative agents of invasive mycoses [5].

Since no fungal vaccines are currently licensed, the only clinical recourse to combat invasive mycoses is the use of antifungal therapeutics (antimycotics). These can be grouped into four different classes on the basis of their mode of action (reviewed in reference [6]): (i) azole derivatives (e.g. miconazole and fluconazole) that inhibit 14α-lanosterol demethylase, which is a key enzyme in ergosterol biosynthesis, a fungal plasma membrane component that does not occur in mammalian cells; (ii) polyene macrolides (e.g. amphotericin B, AmB) that interact with ergosterol and, as such, alter membrane functions; (iii) echinocandins (e.g. caspofungin) that inhibit β-1,3-glucan synthase; (iv) fluorinated pyrimidines (e.g. flucytosine) that inhibit DNA and RNA synthesis. Treatment of invasive mycoses by some of these antimycotics is hampered because of the limited activity spectrum, resistance development, and fungistatic rather than fungicidal activities. Of concern is the prevalence of *Candida* species that display resistance to azoles. Some isolates of *C. glabrata*, for instance, display low fluconazole susceptibility [4]. Moreover, conventional treatment with azoles or AmB is rather ineffective for some emerging fungal pathogens, such as *Fusarium* species [7].

In this review, we will discuss the potential of plant and insect defensins as novel leads for the development of antimycotics. Defensins are part of the host's innate immune system, an ancient defense strategy used by multicellular organisms that include plants, animals, and even fungi to control their natural microbial flora or combat microbial pathogens [8,9,49]. Defensins are small, highly basic cysteine-rich peptides that share a common three-dimensional structure (reviewed in reference [8]). Not only most plant defensins but also the insect defensin heliomicin display antifungal activity *in vitro* and are non-cytotoxic, making them suitable candidate molecules for further antifungal lead optimization [9].

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TABLE 1

Activity of antifungal compounds against different yeast and fungal species

Antifungal class	Compound tested	Survival (%)[a]			MIC (μM)[b]	
		Candida albicans	*Candida krusei*	*Candida glabrata*	*Aspergillus flavus*	*Fusarium solani*
Plant defensins	HsAFP1	0.001	1.8	100	2.5	0.2
	RsAFP2	0.2	8.9	100	0.7	0.04
	DmAMP 1	1.2	100	3.0	>10	0.3
Insect defensins	Heliomicin	0.1	9.0	100	5	2.5
Azoles	Miconazole	0.01	0.07	0.08	0.4	3
	Fluconazole	76.9	100	100	>10	>10
	Itraconazole	65.4	24.1	100	0.4	>10
Polyenes	AmB	<0.01	0.8	0.002	0.4	6

[a] Percentage survival is calculated as the ratio of the amount of colony forming units (CFUs) after 7.5 h incubation of *Candida* spp. (approximately 2×10^5 CFU/mL) at 37 °C with 10 μM of the respective antifungal compounds compared with the amount of CFUs of the DMSO or water-treated cells. Treatment was performed in PDB/YPD (24 g/L PDB; 2 g/L Yeast Extract, Difco; 4 g/L Peptone, Difco; 4 g/L glucose), supplemented with 50 mM HEPES, pH 7.0. The experiment has been repeated twice with similar results.

[b] The minimal inhibitory concentration (MIC) is the concentration of the antifungal that is required to inhibit fungal growth and was calculated from dose–response curves with twofold dilution steps in YPG medium [7] after 24 h incubation at 25 °C.

The plant and insect defensin families consist of over 100 members that display antibacterial and/or antifungal activity, and sometimes insecticidal activity, anti-HIV-1 and/or inhibitory activity against tumor cells [8–12].

In order to identify specific defensins as interesting antifungal leads, their activity spectrum (with emphasis on emerging pathogens and *Aspergillus* sp.) and their mode of action need to be unraveled, and cost-effective production needs to be secured. Here, we will focus on the plant defensins RsAFP2 from radish (*Raphanus sativus*) [13], DmAMP 1 from dahlia (*Dahlia merckii*) [14], and HsAFP1 from coral bells (*Heuchera sanguinea*) [14], and on the insect defensin heliomicin from the tobacco budworm (*Heliothis virescens*) [15].

Spectrum of specific antifungal plant and insect defensins

In order to determine the activity spectrum of plant and insect defensins, their fungicidal or fungistatic activity *in vitro* against *Candida* species, including *C. glabrata*, and antifungal activity against the filamentous fungi *A. flavus* and *F. solani* was assessed (Table 1). To treat mycoses, fungicidal compounds are favored over fungistatic compounds, since the latter may contribute to resistance development [16]. Moreover, in the same experimental setup, the activity spectrum of compounds currently used to treat mycoses, including the azoles miconazole, fluconazole, and itraconazole, and the polyene amphotericin B (AmB) was assessed (Table 1). A compound was considered to be fungicidal if viability of the treated yeast culture was reduced by 99.9% when compared with the control treatment.

The plant defensins HsAFP1 and RsAFP2 and the insect defensin heliomicin were all able to inhibit growth of *C. albicans* and *C. krusei* but not of *C. glabrata*. Since RsAFP2 and heliomicin interact with the same fungal membrane target, glucosylceramides (see below), it is not surprising that their activity spectrum is analogous. Moreover, *C. glabrata* does not synthesize glucosylceramide [17], explaining its resistance to RsAFP2 and heliomicin. HsAFP1 is the only plant defensin that displayed genuine fungicidal activity against *C. albicans*, as did miconazole. However, the nature of the specific HsAFP1-binding sites on membranes of susceptible yeast species still needs to be elucidated. RsAFP2, HsAFP1, and heliomicin displayed *in vitro* activity against both filamentous fungi, with RsAFP2 displaying the strongest activity. The activity spectrum of the plant defensin DmAMP1 differs from the spectrum of the other defensins studied, as DmAMP1 was able to inhibit growth of *C. albicans* and *C. glabrata*, but not of *C. krusei* and *A. flavus*. DmAMP1 interacts with inositolphosphoryl-containing sphingolipids. Whether differences in inositolphosphoryl-containing sphingolipids account for the observed DmAMP1 resistance of some fungal pathogens needs to be investigated. In our experimental setup, only miconazole demonstrated antifungal activity against all pathogens tested. Overall, on the basis of their broad spectrum *in vitro* activity, plant and insect defensins should be considered as valuable candidates for antifungal therapeutics.

Mode of action of antifungal plant and insect defensins

Most cationic antimicrobial peptides induce membrane permeabilization after initial electrostatic binding to negatively charged phospholipids in the plasma membrane. By contrast, RsAFP2, DmAMP1, and HsAFP1 interact with fungus-specific high-affinity plasma membrane binding sites and induce membrane permeabilization [18–20]. The binding sites for RsAFP2, DmAMP1, and heliomicin were identified as fungus-specific sphingolipids:mannosyl diinositolphosphorylceramide ($M(IP)_2C$) for DmAMP1 [21,22] and glucosylceramides (GlcCer) for RsAFP2 and heliomicin [23]. The latter two can be further differentiated upon their binding to different motifs of fungal GlcCer [23]. Interestingly, both RsAFP2 and heliomicin do not interact with human GlcCer, most probably because of a different ceramide structure, explaining their low cytotoxicity [23]. HsAFP1 is unlikely to interact with GlcCer or with $M(IP)_2C$, since yeast deletion mutants defective in biosynthesis of GlcCer or $M(IP)_2C$ have wild-type sensitivity to HsAFP1 (Thevissen *et al.*, unpublished). However, the HsAFP1-binding sites could not yet be identified.

It was recently demonstrated that RsAFP2 induces endogenous reactive oxygen species (ROS) in *C. albicans* but not in an RsAFP2-resistant *C. albicans gcs1*-deletion mutant impaired in GlcCer synthesis (Figure 1) [24]. Moreover, the induction of endogenous ROS by RsAFP2 was found to be causatively linked to its antifungal activity. This suggests that RsAFP2 induces an intracellular signal-

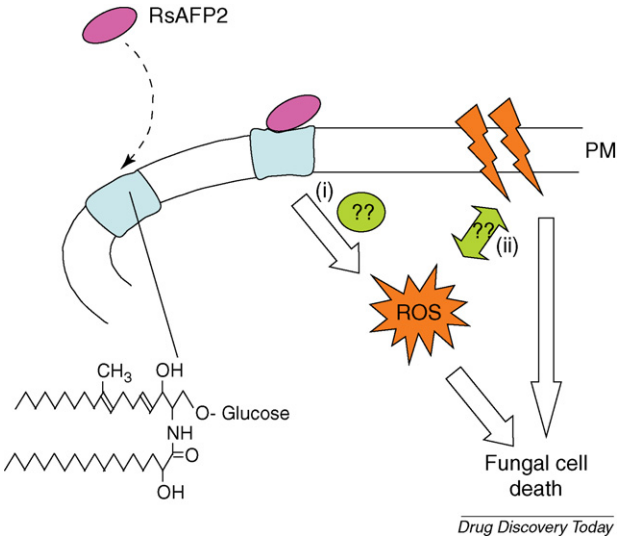

FIGURE 1

Tentative model for the mode of action of RsAFP2, an antifungal plant defensin from radish. RsAFP2 (pink ovals) interacts with glucosylceramides (gray boxes represented by its chemical structure) in the plasma membrane (PM) of susceptible fungi. Upon this initial interaction, membranes are permeabilized (orange arrows), leading to fungal cell death. Additionally, RsAFP2 induces toxic reactive oxygen species (ROS) after its initial interaction with glucosylceramides. It is currently not clear (green question marks) (i) how ROS in susceptible fungi are generated, and (ii) whether there is a functional link between ROS generation and membrane permeabilization.

ing pathway, leading to membrane permeabilization upon initial interaction with GlcCer, rather than merely causing membrane permeabilization by directly inserting into the plasma membrane [24]. ROS are produced as byproducts of aerobic respiration and may damage proteins, lipids, and DNA, resulting in loss of viability [25]. In mammalian cells, an increase in membrane ceramide content can induce ROS production in mitochondria [26]. Whether RsAFP2 induces ROS production through degradation of GlcCer resulting in elevated ceramide levels needs to be investigated further. Since endogenous ROS induction is a typical phenotypic characteristic of apoptosis, or programmed cell death, in yeast [27], plant defensins might induce yeast apoptosis.

Until now, it is unclear whether plant defensins are taken up by fungal cells upon sphingolipid interaction, or whether they stay outside and modulate cellular processes leading to fungal cell death (e.g. ROS production) via their sphingolipid interaction. Only for the pea defensin *Psd1* [28] has cellular uptake been demonstrated, since it was found to interact with a fungal cyclin-like protein that is involved in cell cycle control and co-localized with the nuclei of susceptible fungal cells [29]. It has, however, not been demonstrated that Psd1 interacts with sphingolipids before cellular entry.

Fungal sphingolipids are promising targets for novel antifungals [30]. First, sphingolipids are ubiquitous eukaryotic membrane components yet those found in fungal cells are structurally different from those in human cells. Second, sphingolipids are important pathogenicity determinants in pathogenic yeasts [31–32]. Antifungal compounds that target such pathogenicity determinants and, hence, directly affect infection, can be expected efficiently to combat fungal infections. Theoretically, *in vivo* resistance development is unlikely, since altered sphingolipid composition impacts pathogenicity. Third, sphingolipids appear to play a central role in signal transduction and cell regulation [33]. A central event in these sphingolipid-mediated processes is the generation of ceramides via breakdown of sphingolipids, resulting in cell cycle arrest and apoptosis [34]. Apart from plant defensins and heliomicin, other naturally occurring antifungal peptides have been reported to interact specifically with fungal sphingolipids, including the *Pseudomonas syringae* small antifungal lipopeptide Syringomycin E [35] and the pokeweed antimicrobial protein PaAMP [36]. Moreover, anti-GlcCer antibodies that specifically interact with fungal GlcCer display antifungal activity *in vitro* [37].

Interestingly, the *in vitro* frequency of occurrence of spontaneous RsAFP2-resistant and heliomicin-resistant *C. albicans* mutants (i.e. 1 in 2.10^4) is 5–10 times higher than that of DmAMP1-resistant mutants (i.e. 1 in 10^5) and at least 100 times higher than that of HsAFP1-resistant *C. albicans* mutants (i.e. less than 1 in 2.10^6) (Thevissen *et al.*, unpublished). Possibly, HsAFP1 interacts with essential fungal plasma membrane structures [18]. However, the observed high *in vitro* resistance frequency for RsAFP2 and heliomicin does not necessarily imply low *in vivo* performance since, as mentioned, they target pathogenicity factors.

Regarding tolerance mechanisms of fungal pathogens against plant or insect defensins, the involvement of intracellular signaling pathways has recently been reported [38]. More specifically, tolerance of susceptible fungi toward RsAFP2 is mediated by the Gpmk and Mgv1 MAPK signaling cascades [38]. Both Gpmk1 and Mgv1 belong to a family of serine/threonine protein kinases that activate transcription factors involved in regulating multiple developmental processes related to cell wall integrity, sexual reproduction, and pathogenicity [39].

Lead optimization

For RsAFP2 and heliomicin, improved variants have been designed for lead optimization. Mutational analysis of RsAFP2 was performed on the basis of sequence comparison of RsAFP2 with the near identical, but less active, radish defensin RsAFP1. Since the cationicity of RsAFP2 is higher than that of RsAFP1 [13], an increase in cationicity of RsAFP2 by directed replacement of specific amino acid residues with arginine was speculated to enhance further the antifungal activity [40]. Indeed, two substitution variants, namely RsAFP2(G9R) and RsAFP2(V39R) in which a glycine or valine at positions 9 and 39, respectively, were replaced by an arginine (Table 2) were more active against *Fusarium culmorum* than wild-type RsAFP2 [40]. To select candidate amino acid residues for substitution in heliomicin, the sequence of heliomicin was compared with the sequence of the ARD1 defensin, isolated from caterpillars of the Lepidoptera *Archeoprepona demophoon* [7]. Although ARD1 differs from heliomicin by only two residues, ARD1 is twofold to eightfold more active against *A. fumigatus* and *C. albicans*. Mutational analysis of ARD1 by replacement of an asparagine on position 19 by an arginine (ETD-151, Table 2), resulting in an increased cationicity, or by replacement of alanine on position 36 by leucine (ETD-135, Table 2), resulting in increased hydrophobicity, enhanced ARD1 activity against *C. albicans* and *A. fumigatus* twofold to fourfold [7]. In addition, these variants were active against opportunistic filamentous fungal pathogens such as *F. solani* and *Scedosporium prolificans*, for which currently no effective treatment exists. Hence, in general, activity of an

TABLE 2

Comparison of the complete amino acid sequence of various plant and insect defensins and their synthetic variants

Defensin	Reference	Sequence
RsAFP2	[13]	--QKLCQRPSGTWSGVCGNNNACKNQCIRLEK-ARHGSCNYVFPAHKCICYFPC
RsAFP2(V39R)	[40]	--QKLCQRPSGTWSGVCGNNNACKNQCIRLEK-ARHGSCNY**R**FPAHKCICYFPC
RsAFP2(G9R)	[40]	--QKLCQRPS**R**TWSGVCGNNNACKNQCIRLEK-ARHGSCNYVFPAHKCICYFPC
Heliomicin	[15]	--DKLIG--SCVW-GAVNYTSDCNGECKR-RG-YKGGHCGS-FANVNCWCET
ARD1	[7]	--DKLIG--SCVW-GAVNYTS**N**CN**A**ECKR-RG-YKGGHCGS-FANVNCWCET
ETD-151	[7]	--DKLIG--SCVW-GAVNYTS**N**C**RA**ECKR-RG-YKGGHCGS-FANVNCWCET
ETD-135	[7]	--DKLIG--SCVW-GAVNYTS**N**CN**A**ECKR-RG-YKGGHCGS-F**L**NVNCWCET
DmAMP1	[14]	---ELCEKASKTWSGNCGNTGHCDNQCKSWEG-AAHGACHVRNGKHMCFCYFNC
HsAFP1	[14]	DGVKLCDVPEGTWSGHCGSSSKCSQQCKDREHFAYGGACHYQFPSVKCFCKRQC

Residues conserved between RsAFP2 and heliomicin and their variants are in gray; cysteines are in yellow and mutated amino acids in the analogs are in blue. Boxes represent secondary structure elements.

antifungal peptide that interacts with target membranes can be improved by increasing the cationicity and/or hydrophobicity.

Heterologous peptide production systems

Commercial utilization of peptides requires stable production to secure the required amounts of adequate quality and purity. In principle, both chemical synthesis and recombinant production systems can be utilized. However, owing to the relatively large size of defensins (40–60 amino acids), their higher order tertiary structure, and presence of 3–4 disulfide bridges, only recombinant production is commercially viable. For research purposes, antifungal peptides have been heterologously expressed in diverse hosts, such as bacteria, yeasts, fungi, and plants.

Escherichia coli expression utilizes fusion protein strategies to produce the soluble or insoluble peptides in the cytoplasm [41–44]. A frequently employed system is the pET/Origami™ system (www.novagen.com), in which the *E. coli* Origami™ host strain has mutations in both the thioredoxin reductase and the glutathione reductase genes to enhance greatly cytoplasmic disulfide bond formation [43]. Recently, correctly folded and matured drosomycin has been produced in *E. coli* as a glutathione *S*-transferase-fusion protein, albeit with peptide yields less than 1 mg/L [44]. The major advantages of the *E. coli*-based systems are the wealth of commercially available strains and plasmids, its genetic amenability, and short generation time.

Expression of antifungal defensins in fungi has mainly been performed in the yeasts *Saccharomyces cerevisiae* and *Pichia pastoris* and, to a lesser extent, in filamentous fungi, such as *Aspergillus* spp. The major advantages of fungal production include the potential for high-level production (multiple grams per liter), secreted production facilitating downstream processing and purification, and correctly matured and folded product with formation of the essential disulfide bridges. The obvious disadvantage of fungal production is the inherent sensitivity of the host toward the peptides with antifungal activity. However, this may be overcome using resistant hosts that either occur naturally or are generated by modification of biosynthetic pathways to alter, or eliminate, the specific fungal target(s), such as sphingolipids. Both *S. cerevisiae* and *P. pastoris* have been approved by FDA and EMEA as safe hosts for pharmaceutical production while products from *Aspergillus* have GRAS (generally recognized as safe) status, allowing them to be used in the food and feed industry. No pharmaceutical peptides produced by *Aspergillus* have been approved yet, but some are entering phase III clinical trials (e.g. talactoferrin, www.agennix.com).

For yeast expression, defensin cDNAs are often fused to the MFα1 signal sequence and pro-region, while separating the MFα1 pro-region and the mature peptide by a KR dipeptide that is processed by the Kex2 protease. In this way, four distinct drosomycin-related peptides were produced, three of which represented aminoterminal extended peptides [45]. The correctly matured drosomycin accounted for only 25% of the total amount of drosomycin-related peptides. However, upon co-expression of the Kex2 protease, the correctly processed drosomycin increased to 90% of the total amount, with a total yield of 2 mg/L [46]. Also the *Pisum sativum* defensin Psd1 has been produced in *P. pastoris*, using the MFal signal sequence and pro-region, resulting in a yield of up to 60 mg/L [47].

Expression systems based on filamentous fungi, such as *Aspergillus* and *Fusarium*, are superior to yeast-based systems with expression levels up to 10s of g/L in the most favorable cases [48]. Plectasin, a fungal antimicrobial defensin from *Pseudoplectania nigrella* [49], has been produced in a proprietary fungal *Aspergillus oryzae-based* production system [50]. This system is currently used for industrial scale, cost-effective production of other proteins, such as amylases, proteases, glucanases, oxidoreductases, and lipases [48,51] and employs shuttle vectors and ectopic integration that allow multicopy presence of the genes of interest. Strong and maltose-inducible amylase promoters are used to drive heterologous expression [50]. As in the yeast-based systems, the pro-region is separated from the mature peptide by a Kex2 protease site. Currently, a derivative of plectasin with enhanced antimicrobial potency is in preclinical development for use against bacterial (Gram-positive) infections caused by Staphylococci and Streptococci.

Apart from the above-described microbial heterologous expression systems, plant defensins have also been heterologously produced in plants such as *Arabidopsis thaliana* [52–53]. The genes encoding DmAMP1 and RsAFP2 have been expressed under control of the constitutive Cauliflower Mosaic Virus 35S promoter [52], leading to low production levels of 0.09% of total soluble proteins. However, expression of the two genes as a chimeric polyprotein, consisting of the two mature protein domains separated by a 16-amino acid linker peptide, caused a significant increase in expression level leading to 0.62% of total soluble proteins [52].

Both heterologously produced plant defensins retained their bioactivity. Arabidopsis plant defensins and the human beta-defensin-2 [54,55] were produced in post-transcriptional gene silencing impaired Arabidopsis mutants using a transformation vector containing matrix attachment regions [56]. Significant amounts of bioactive peptides (up to 3% of total soluble proteins) were obtained. Besides Arabidopsis, *Nicotiana benthamiana* has also been used for heterologous defensin production using a viral expression system [57].

In vivo performance of defensins against fungal infections

Owing to a generally poor correlation between *in vitro* and *in vivo* activities of antimycotics, which may be accounted for by factors such as drug pharmacokinetics, drug delivery to the site of infection, treatment of the site of infection, (lack of) host response, and production of toxins, animal models may never be totally replaced [58,63]. With *C. albicans* as target organism for antifungal drug evaluation, different *in vivo* evaluation levels (primary, secondary, tertiary) should be considered. As a primary evaluation, vaginal candidiasis in mouse is most suitable and involves topical treatment for a topical infection. This approach, theoretically, is the most sensitive and straightforward, as topical treatment excludes drug absorption and metabolism phenomena. In a secondary evaluation, different infective doses can be administered intravenously and antifungal compounds are given intraperitoneally, thereby using survival in acute infection and *Candida* burdens in target organs (liver, spleen, lung, brain, and skin) in the case of chronic infection as readout. For promising antifungal compounds, a tertiary evaluation may include the acute systemic candidiasis model in Guinea pigs, which are more sensitive to fungal infections and may better reflect the situations that occur in man.

Regarding animal models for *Aspergillus* infections, birds are natural hosts of *A. fumigatus*. Furthermore, rabbits are also highly susceptible toward *A. fumigatus* infections, followed by mice, rats, and Guinea pigs [58]. Rabbits have the advantage of allowing multiple sampling on the same animal, better clinical evaluations, and the opportunity to obtain body fluids, such as cerebrospinal fluid (CSF), that are not readily obtained in sufficient quantity from mice [59]. On the contrary, mice are easy to handle in constant laboratory use and *Aspergillus* infections can be established in various ways: systemic infection in noncompromised mice, cerebral infection in pancytopenic mice, and pulmonary infection in steroid-suppressed mice. Also, inhalational murine models can be used to address *in vivo* efficacy of antifungal drugs [59]. Regarding the intravenous, intranasal/inhalative, and intraabdominal routes of *Aspergillus* infections in mice, all stereotypically result in an infection process that is mainly restricted to the kidneys as the main target organ [58].

Although at present only limited data are available, one study that addresses the *in vivo* performance of an insect defensin against fungal infections in an animal model has been reported. The heliomicin variant EDT151 (Table 2) performed well *in vivo* in mice infected with *Candida* sp. and *Aspergillus* sp. when compared with AmB and various azoles, including ketoconazole, fluconazole, and itraconazole [60]. In addition, EDT151 was characterized by very low toxicity, even upon intravenous administration [60], making it a suitable candidate peptide for further clinical studies. Although no studies on/the *in vivo* performance of plant defensins have been reported yet, the performance of RsAFP2 on systemic *Candida* infections in mice is currently assayed. Future studies will have to confirm *the* observed *in vivo* efficacy of RsAFP2 against these systemic *C. albicans* infections.

Conclusions

Defensins are ubiquitous components of the innate immune system of diverse multicellular organisms [8,9]. In these different organisms, this immune system similarly acts in the self-defense against microbial invaders that, though the hosts are very different, often employ similar infection strategies [61,62]. Therefore, defense components produced in an organism belonging to one biological kingdom may affect microbial pathogens of hosts belonging to another kingdom [62]. Indeed, heterologous expression of the human defensin HBD2 in plants resulted in an increased resistance to a fungal disease [55]. Vice versa, plant defensins are promising novel leads for antifungal therapeutics in human/animals because, in contrast to many biological antimicrobial peptides, their mode of action involves the highly specific attachment to fungal cells explaining their low cytotoxicity. With their efficient heterologous production, another hurdle in antifungal lead optimization of plant and insect defensins is overcome and the first steps to evaluate their *in vivo* performance have now been taken. So far, plant sciences have contributed to drug development through the discovery of bioactive compounds that are used to treat infections or other diseases, such as the anti-inflammatory aspirin or the anticancer agent taxol [62]. Possibly, plant defensins are the next hallmark in the contribution of plants to medical sciences.

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