Amino Acids
DOI 10.1007/s00726-010-0622-3

REVIEW ARTICLE

# Antimicrobial peptides from *Phyllomedusa* frogs: from biomolecular diversity to potential nanotechnologic medical applications

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Received: 3 February 2010 / Accepted: 5 May 2010


**Abstract** Screening for new bioactive peptides in South American anurans has been pioneered in frogs of the genus *Phyllomedusa*. All frogs of this genus have venomous skin secretions, i.e., a complex mixture of bioactive peptides against potential predators and pathogens that presumably evolved in a scenario of predator–prey interaction and defense against microbial invasion. For every new anuran species studied new peptides are found, with homologies to hormones, neurotransmitters, antimicrobials, and several other peptides with unknown biological activity. From Vittorio Erspamer findings, this genus has been reported as a "treasure store" of bioactive peptides, and several groups focus their research on these species. From 1966 to 2009, more than 200 peptide sequences from different *Phyllomedusa* species were deposited in UniProt and other databases. During the last decade, the emergence of high-throughput molecular technologies involving de novo peptide sequencing via tandem mass spectrometry, cDNA cloning, pharmacological screening, and surface plasmon resonance applied to peptide discovery, led to fast structural data acquisition and the generation of peptide molecular libraries. Research groups on bioactive peptides in Brazil using these new technologies, accounted for the exponential increase of new molecules described in the last decade, much higher than in any previous decades. Recently, these secretions were also reported as a rich source of multiple antimicrobial peptides effective against multidrug resistant strains of bacteria, fungi, protozoa, and virus, providing instructive lessons for the development of new and more efficient nanotechnological-based therapies for infectious diseases treatment. Therefore, novel drugs arising from the identification and analysis of bioactive peptides from South American anuran biodiversity have a promising future role on nanobiotechnology.

**Keywords** *Phyllomedusa* · Bioprospection · Antimicrobial peptide · Dermaseptin · Infection disease · New drugs · Nanobiotechnology

**Abbreviations**

| | |
|---|---|
| ADR | Adenoregulin |
| AFM | Atomic force microscopy |
| AMP | Antimicrobial peptide |
| CD | Circular dichroism |
| DRP | Dermaseptin related peptide |
| DRS | Dermaseptin |

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Published online: 05 June 2010

| | |
|---|---|
| DRT | Dermatoxin |
| FSAP | Frog skin active peptide |
| FTIR | Fourier-transformed infrared spectroscopy |
| HIV-1 | Human immunodeficiency virus 1 |
| HSV-1 | Herpes simplex virus 1 |
| MALDI | Matrix assisted laser desorption ionization |
| NMR | Nuclear magnetic resonance |
| NPY | Neuropeptide Y |
| PLS | Phylloseptin |
| PLX | Phylloxin |
| PM | Plasmatic membrane |
| PTC | Plasticin |
| PYY | Polypeptide YY |
| SPYY | Skin polypeptide YY |
| UniProt | Universal protein resource |

## South America Phyllomedusae biodiversity

According to Frost, up to now, over 5,600 anuran species have been described around the world, in a wide variety of environments, except in the poles (Frost 2009). Compared to other continents, South America includes the highest number of the anuran species of the world in its biomes (Toledo and Jared 1995). Brazil (821 spp.) (SBH 2009), Colombia (732 spp.) (Frost 2009), and Ecuador (480 spp.) (Coloma 2009) are the richest countries in anurans' species in South America.

Among all South American anurans, a complex group of 32 valid species of *Phyllomedusa* frogs (Table 1) deserve special attention. The *Phyllomedusa* genus belongs to the subfamily Phyllomedusinae (Amphibia, Anura, Hylidae) that has seven genera of neotropic tree frogs distributed from Central America to the east Andes along South America (*Agalychnis*, *Cruziohyla*, *Hylomantis*, *Pachymedusa*, *Phasmahyla*, *Phrynomedusa*, and *Phyllomedusa*). The *Phyllomedusa* species display unique characters among the neotropical hylidaes, including vertical slit pupil, green back, hidden regions with contrasting patterns of red, blue, and yellow (Caramaschi 2006). Eggs deposited out of water give rise to aquatic larvae with different exclusive characters, in addition to 95 transformations in nuclear and mitochondrial proteins and ribosomic genes (Faivovich et al. 2005).

According to Caramaschi (2006), most of the *Phyllomedusa* species are distributed among five groups: *P. burmeisteri* group, *P. hypochondrialis* group, *P. buckleyi* group, *P. perinesos* group, and *P. tarsius* group (Caramaschi 2006). There are, however, some species that are currently not assigned to any group, such as *P. atelopoides*, *P. bicolor*, *P. coelestis*, *P. palliata*, *P. tomopterna*, *P. trinitatis*, *P. vaillantii*, and *P. venusta* (Caramaschi 2006).

The species belonging to the *Phyllomedusa* genus are frequently renamed by herpetologists. These changes influence other science areas that depend on the correct taxonomic identification, mainly the "omics" sciences, such as proteomics, peptidomics, secretomics, genomics, and others, leading to new molecules described in discontinued, invalid, or non-described species names.

## The *Phyllomedusa* cutaneous glands

The anuran skin displays great morphofunctional diversity adapted to a number of adverse factors present in the species habitat environment (Toledo and Jared 1993; Barra and Simmaco 1995). The cutaneous glands present in the skin play an essential role in respiration, reproduction, defense against predators and protection against desiccation, and proliferation of microorganisms on the body surface (Toledo and Jared 1995). Ultrastructural characterization of the *Phyllomedusa* species skin demonstrated that the profile of skin glands are composed by three types of cutaneous glands (acinous) differed in size and secretory activity. These are lipid, mucous, and serous glands that lie deep in the skin and subcutaneous connective tissue (Lacombe et al. 2000).

The lipid glands promote the impermeabilization of the skin in order to decrease water loss (Castanho and De Luca 2001). The mucous glands produce mucus to support cutaneous functions, such as respiration, reproduction, thermoregulation, and defense (Toledo and Jared 1995). The serous glands are the largest and are widely distributed over the animal's body surface, as a main element in amphibian passive defense (Toledo and Jared 1995; Lacombe et al. 2000). These glands produce a wide variety of noxious or toxic substances with various pharmacological effects on microorganisms, vertebrate, and invertebrate species (Toledo and Jared 1995; Lacombe et al. 2000). The serous glands exhibit remarkable polymorphism in *Phyllomedusa* (Delfino et al. 1998). They are classified basically into two classes, type I and II (Lacombe et al. 2000).

Type I glands exhibit a poorly developed smooth endoplasmic reticulum (Lacombe et al. 2000) and present two subtypes, Ia and Ib. Type Ia shows dense granules which characterize the biosynthesis of proteinaceous products reserved for exocytosis, and engage both rough endoplasmic reticulum and Golgi apparatus (Delfino 1991). Type Ib shows vesicles holding a lucent material in the fluid serous secretions on the anuran skin, which undergo maturation without condensation (Toledo and Jared 1995).

Type II glands, typical of *Phyllomedusa bicolor*, show a well-developed smooth endoplasmic reticulum that is possibly engaged in the biosynthesis of peptides (Blaylock et al. 1976; Lacombe et al. 2000). These peptides are

**Table 1** Up to date list of *Phyllomedusa* species distributed by group and number of peptides characterized

| Group | Species | Number of peptides | References |
|---|---|---|---|
| *P. burmeisteri* [5 species] | *P. bahiana* | — | |
| | *P. burmeisteri* | 29 | Barra et al. (1994), Mandel (2008), Mundim (2008), UniProt (2009) |
| | *P. distincta* | 6 | Batista et al. (1999, 2001) |
| | *P. iheringii* | — | |
| | *P. tetraploidea* | — | |
| *P. hypochondrialis* [11 species] | *P. araguari* | — | |
| | *P. azurea* | 41 | Thompson (2006), Thompson et al. (2006), Thompson et al. (2007a, b), UniProt (2009) |
| | *P. ayeaye* | — | |
| | *P. centralis* | — | |
| | *P. hypochondrialis* | 34 | Leite et al. (2005), Brand et al. (2006a, b), Chen et al. (2006), Conceição et al. (2006, 2007), UniProt (2009) |
| | *P. itacolomi* | — | |
| | *P. megacephala* | — | |
| | *P. nordestina* | 3 | Conceição et al. (2009) |
| | *P. oreades* | 6 | Brand et al. (2002), Leite et al. (2005) |
| | *P. palliata* | — | |
| | *P. rohdei* | 22 | Anastasi et al. (1966), Barra et al. (1985), Montecucchi et al. (1986), Mandel (2008), Mundim (2008) |
| *P. perinesos* [4 species] | *P. baltea* | — | |
| | *P. duellmani* | — | |
| | *P. ecuatoriana* | — | |
| | *P. perinesos* | — | |
| *P. tarsius* [5 species] | *P. boliviana* | — | |
| | *P. camba* | — | |
| | *P. neildi* | — | |
| | *P. sauvagii* | 31 | Anastasi et al. (1969), Montecucchi et al. (1979), Montecucchi et al. (1981a), Montecucchi et al. (1981b), Erspamer et al. (1985), Richter et al. (1987), Mor et al. (1991a, b), Chen et al. (2003a, b), Mor and Nicolas (1994a), Chen and Shaw (2003), Chen et al. (2005a), UniProt (2009) |
| | *P. tarsius* | 12 | Silva et al. (2000), UniProt (2009) |
| Unassigned to group [7 species] | *P. atelopoides* | — | |
| | *P. bicolor* | 21 | Anastasi et al. (1970), Richter et al. (1990), Daly et al. (1992), Mignogna et al. 1992, Amiche et al. (1993), Amiche et al. (1994), Mor et al. (1994a, b); Charpentier et al. (1998); Fleury et al. (1998), Seon et al. (2000), Amiche et al. (2000), Pierre et al. (2000), Vanhoye et al. (2003), Chen et al. (2005b), UniProt (2009) |
| | *P. coelestis* | — | |
| | *P. tomopterna* | 21 | Mandel (2008), Mundim (2008), UniProt (2009) |
| | *P. trinitatis* | 1 | Marenah et al. (2004) |
| | *P. vaillantii* | — | |
| | *P. venusta* | — | |

synthesized as prepropeptides that are processed into mature peptides after removal of the peptide signal and the acidic propiece. These are then stored in the granules (Nicolas and El Amri 2009). It is proposed that the release of the gland content onto the skin surface is mediated by a holocrine mechanism involving rupture of the plasmatic membrane (PM) and extrusion of the granules through a duct opening to the surface (Nicolas and El Amri 2009).

Immunofluorescence analysis of the *P. bicolor* dermal glands using an antibody to the acidic propiece region of the preprodermaseptin/preprodeltorphins-derived family [ENENEENHEEGSE] demonstrated that the fluorescence-positive reaction is restricted to the serous glandular content, indicating their specificity in the biosynthesis and secretion of dermaseptins and deltorphin peptides (Lacombe et al. 2000). A recent mass spectral image study (MALDI-image) of the skin of *P. hypochondrialis* indicates that the serous glands present specialization in the peptide production and storage (Brand et al. 2006b).

## Peptides of *Phyllomedusa* skin secretions

In spite of the large number of anuran species from different genera found within South America, a great deal of attention is being paid to the study of neotropical hylid frogs that belong to the subfamily *Phyllomedusinae*, as an excellent source of these molecules. Erspamer et al. (1985) also stated that "No other amphibian skin can compete with that of the Phyllomedusae". The initial efforts on *Phyllomedusa* skin secretions by Vittorio Erspamer followed by other scientists around the world during the last four decades revealed a complex cocktail of biologically active peptides with antimicrobial, hormonal, and neuro activities (Bevins and Zasloff 1990; Amiche et al. 1993). The peptides secreted differ significantly among species within this genus leading to an interesting molecular diversity, associated with possible specific differences present in the specie niche, such as the interactions with environment, predators, and pathogens characterizing *Phyllomedusa* species evolution.

The first peptide isolated from the *Phyllomedusa* skin was Phyllokinin [RPPGFSPFRIY], a bradykinyl-isoleucyl-tyrosine *O*-sulfate from *P. rohdei* in 1966 (Anastasi et al. 1966), followed by Phyllocaerulein [QEYTGWMDF-$NH_2$] a cerulein-like nonapeptide from *P. sauvagii* in 1969 (Anastasi et al. 1969). All these bioactive peptides were discovered by Erspamer's research group. Due to technical limitations, large numbers of specimens have to be killed in order to isolate, characterize, and perform the biological assays on the two peptides. Since the 1960s, the number of *Phyllomedusa* peptides discovered has increased exponentially (Fig. 1, inset) followed by the drastic reduction of specimens required for the analyses. Nowadays, it is possible to carry out transcriptome analysis to build a cDNA library only with the secretions from a single living specimen (Chen et al. 2003b). The impacts caused by the bioprospecting activity on the frog natural populations tend to zero through the development of non-invasive techniques largely due to scientific and technical advances.

The emergence of modern high-throughput molecular technologies involving de novo peptide sequencing via

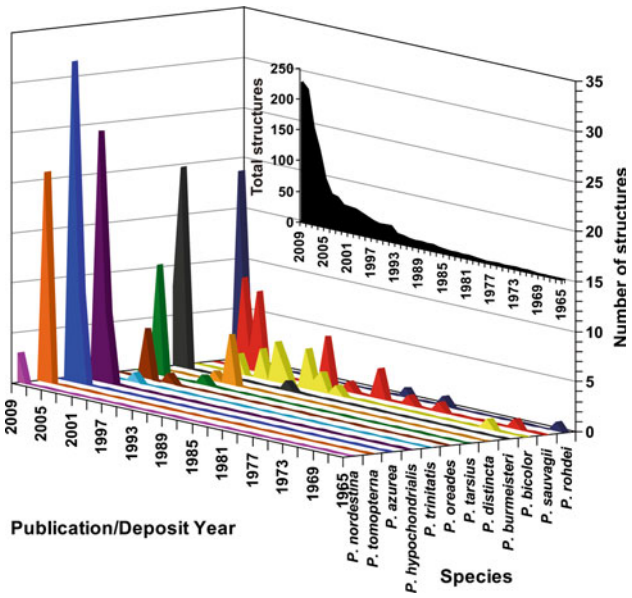


**Fig. 1** *Phyllomedusa* peptides and prepropeptides amino acid sequences published on indexed scientific journals including the structures deposited in genomic and proteomic databases from 1965 to 2009. *Inset* number of *Phyllomedusa* primary structures increment per year showing an exponential growth

tandem mass spectrometry, cDNA cloning, and pharmacological screening applied to peptide discovery allowed fast structural data analysis and the generation of peptide sequence libraries, which in turn increased the capacity of peptide characterization, remarkably reducing the amount of samples needed (Shaw 2009).

The chronology related to the analyses of the *Phyllomedusa* peptide discovery (Fig. 1) was impacted by the technological evolution applied to the study of venom-derived peptides, including the emergence of new research groups dedicated to the characterization of anuran venoms.

From 1966 to 2009, 227 peptide amino acid sequences, including peptide precursor cDNA sequences, belonging to the frog skin active peptide (FSAP) family from the skin of *Phyllomedusa* species (Fig. 1, inset) were published in scientific papers and/or deposited on genomic and/or proteomic data banks as the Universal Protein Resource Consortium (UniProt). The species *P. azurea*, *P. bicolor*, *P. burmeisteri*, *P. distincta*, *P. hypochondrialis*, *P. nordestina*, *P. oreades*, *P. rohdei*, *P. sauvagii*, *P. tarsius*, *P. tomopterna*, and *P. trinitatis* that belong to all groups, except the *P. perinesos* group, had their secreted peptides sequenced by 2009 (Table 1).

The *Phyllomedusa* skin peptides are grouped in to three main groups according to their "primary" activity: antimicrobial peptides (AMPs); smooth muscle active peptides; and nervous system active peptides (Table 2) (Erspamer et al. 1981). However, peptides' secondary activities were not considered in this systematization. The

**Table 2** Skin peptide families, main activity, and distribution on *Phyllomedusa* species

| Main activity | Peptide family | Species | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *P. azurea* | *P. bicolor* | *P. burmeisteri* | *P. distincta* | *P. hypochondrialis* | *P. nordestina* | *P. oreades* | *P. rohdei* | *P. sauvagii* | *P. tarsius* | *P. tomopterna* | *P. trinitatis* |
| Antimicrobial | *Dermaseptin* (Mor et al. 1991a) | X | X | X | X | X | | X | X | X | X | X | X |
| | *Dermatoxin* (Amiche et al. 2000) | | X | | | | | | | X | X | | |
| | *Distinctin* (Batista et al. 2001) | X | | X | X | | | | | | | | |
| | *Phylloseptin* (Leite et al. 2005) | X | X | X | | X | | X | X | | X | X | |
| | *Phylloxin* (Pierre et al. 2000) | | X | | | | | | | X | | | |
| | *Plasticins* (Vanhoye et al. 2004) | | X | | | | | | | X | | | |
| | *SPYY* (Mor et al. 1994a, b) | | X | | | | | | | | | | |
| Central nervous system active | *Deltorphin* (Erspamer et al. 1989) | | X | X | | | | | | X | | | |
| | *Dermorphin* (Broccardo et al. 1981) | | | | | X | | | X | X | | | |
| Smooth muscle active | *Bradykinin* (Brand et al. 2006a) | X | | X | | X | X | | X | X | | X | |
| | *Phyllokinin* (Anastasi et al. 1966) | X | | | | X | | | X | X | | | |
| | *Tryptophyllin* (Gozzini et al. 1985) | X | | | | X | | | X | | | | |
| | *Litorin* (Barra et al. 1985) | | | | | | | | X | | | | |
| | *Phyllolitorin* (Yasuhara et al. 1983) | | | | | | | | | X | | | |
| | *Phyllomedusin* (Anastasi and Erspamer 1970) | | X | | | | | | | | | | |
| | *Phyllocaerulein* (Anastasi et al. 1969) | | | | | | | | | X | | | |
| | *Sauvagine* (Montecucchi et al. 1980) | | | | | | | | | X | | | |
| | *Sauvatide* (Wang et al. 2009) | | | | | | | | | X | | | |
| | *S-Calcitonin* gene related (Seon et al. 2000) | | X | | | | | | | | | | |
| Unknown | *Hyposin* (Thompson et al. 2007b) | X | | | | | | | X | | | | |

first group acts as a skin anti-infective passive defense barrier, the second and the third groups cause the disruption of the predator homeostasis balance. The biological activity of the hyposin peptides is still unknown.

## Antimicrobial peptides

Among the peptides of the FSAP family, the AMPs are the most diverse class. To date, the AMPs described in *Phyllomedusa* skin include seven distinct families (or subfamilies) according to their sequence similarity and activity, e.g., *Dermaseptin* (Mor et al. 1991a), *Dermatoxin* (Amiche et al. 2000), *Distinctin* (Batista et al. 2001), *Phylloseptin* (Leite et al. 2005), *Phylloxin* (Pierre et al. 2000), *Plasticin* (Vanhoye et al. 2004), and *Skin Polypeptide YY* (Mor et al. 1994a, b, c). These peptides comprise the skin anti-infective passive defense barrier of these anurans. According to Pierre et al. (2000), the biological significance of such a complex mixture of antibiotic peptides with different specificity and potency in *Phyllomedusa* skin is possibly related to a greater protection against a wide range of potential invaders at a minimum metabolic cost (Pierre et al. 2000), e.g., dermaseptins exhibit synergy of action upon combination with other antibiotic molecules or AMPs, resulting in a 100-fold increase in antibiotic activity potency (Mor and Nicolas 1994b; Giacometti et al. 2006).

These peptides differ in amino acid composition, length, structure, specificity, and several other non-antimicrobial activities, but share common physico-chemical properties, such as cationic charge and an amphipathic structure when interacting with PMs. They have also shared a conserved prepro region originating from a single gene family named preprodermorphin/dermaseptins-derived peptides family that unites them (Nicolas and El Amri 2009). This canonical precursor (Table 3) has an architecture that comprises: a N-terminal pre-sequence composed by the signal peptide, with the first 22 amino acid residues; the acidic propiece with 21–24 residues in the middle region, that terminates in a typical -KR- propeptide convertase processing motif that cleaves and releases each respective mature peptide located at the C-terminus (Chen et al. 2005a) with remarkably conserved, both within and between species; and a markedly different C-terminal domain sequence corresponding to chemically and functionally different mature peptides with 19–34 residues that include amphipathic antimicrobial peptides as well as dermorphins and deltorphins, D-amino acid-containing heptapeptides which are very potent and specific agonists of the $\mu$-opiod or $\delta$-opioid receptors (Erspamer 1992; Amiche et al. 1998, 1999; Lazarus et al. 1999; Pierre et al. 2000; Nicolas et al. 2003; Vanhoye et al. 2003; Nicolas and El Amri 2009).

Despite the intensive studies, the complete and precise structure–activity relationships and mechanisms of the AMPs action are still not fully understood (Nicolas and El Amri 2009). Morphological and functional assays confirm that PM permeabilization is achieved by distortion of the PM structure, not by activation of a pre-existing pore or transporter (Rivas et al. 2009). The ensuing effects depend on the antimicrobial peptide and the severity of the damage, and usually include dissipation of ionic gradients across the PM, leakage of nutrients and/or larger cytoplasmic components, and finally, a collapse of the parasite bioenergetics and osmotic lysis. This killing mechanism acts promptly by destroying their PM, promoting the reduction of log orders of pathogens in a few minutes (Feder et al. 2000). This mechanism is unlikely to induce antibiotic-resistance in microorganisms due to a great metabolic change in the PM composition (Shai 1995).

Two elements seem to be relevant to the antimicrobial action: the selectiveness, and the ability to destabilize PMs (Hwang and Vogel 1998; Dathe and Wieprecht 1999; Shai 2002; Yeaman and Yount 2003). The biochemical and biophysical properties of the peptide, e.g., amphipathicity, charge, conformation, hydrophobicity, and polar angle, result from the interrelationship between the physico-chemical properties of the amino acid composition and its position in the sequence, driving the peptide three-dimensional configuration (Yeaman and Yount 2003). Therefore, changes in composition, sequence, and intramolecular bonds may profoundly affect the structure–activity relationships of the solubilized AMPs, upon binding to target PMs. The coordination of these events allow the optimization of antimicrobial peptide efficacy determined by the balance between increased affinity against a microbial target versus reduced toxicity to host cells (Matsuzaki 2009).

Matsuzaki (2009) stated that strong antimicrobial activity and less cytotoxicity could be achieved by increasing the net positive charge of the peptide with minimal hydrophobicity above a threshold, which is supported by the hypothesis that the lipid composition of cell surfaces primarily determines cell selectivity. The hydrophobicity responsible for cytotoxicity is displayed by the hydrophobic face of the amphipathic secondary structure formed upon binding to the PM. Residues close to the ends of a helix do not fully contribute to the effective hydrophobicity (Matsuzaki 2009).

According to Nicolas and El Amri (2009), the peptide antimicrobial potency is essentially independent of the bacterial envelope structure, related to the AMP selectiveness. Ultrastructural studies performed by electron microscopy and immunocytochemistry (Hernandez et al. 1992) and also biophysical studies with liposomal models (Pouny et al. 1992) demonstrated that dermaseptin exerts its action through selective lysis of PMs (Mor and Nicolas

**Table 3** Primary structures of *Phyllomedusa* antimicrobial prepropeptides

| Name[a] | | Abbreviation[a] | Prepropeptide Sequence[b] | Species | Digital abstract |
|---|---|---|---|---|---|
| Dermaseptin | AZ3 | DRS-AZ3 (DMS3) | MAFLKKSLFLVLFLGMVSLSIC **EEEKRENEDEEK-Q-EDDEQSEM *KR*** *GLWSTIKNVAAAAGKAALGALGEQ* | *P. azurea* | Q17UY8 (http://www.uniprot.org/uniprot/Q17UY8.html) |
| | AZ4 | DRS-AZ4 (DMS4) | MAFMKKSLFLVLFLGMVSLSIC **EEEKRENEDEAK-Q-EDDEQSEM *KR*** *GLWSTIKNVGKEAAIAAGKAALGALGEQ* | | Q1EJP4 (http://www.uniprot.org/uniprot/Q1EJP4.html) |
| | AZ5 | DRS-AZ5 (DMS5) | ----KSLFLVLFLGMVSLSIC **EEEKRENEDEEK-Q-EDDEQSEM *KR*** *GLWSTIKNVGKEAAIAAGKAVLGSLGEQ* | | Q1EJP5 (http://www.uniprot.org/uniprot/Q1EJP5.html) |
| | B1 | DRS-B1 | MDILKKSLFLVLFLGLVSLSIC **EEEKRENEDEEK-Q--DDEQSEM *KR*** *AMWKDVLKKIGTVALHAGKAALGAVADTISQGEQ* | *P. bicolor* | P80282 (http://www.uniprot.org/uniprot/P80282.html) |
| | B2 | DRS-B2 (ADR) | MAFLKKSLFLVLFLGLVSLSIC **EEEKRENEDEEE-Q-EDDEQSEM *KR*** *GLWSKIKEVGKEAAKAAAKAAGKAALGAVSEAVGEQ* | | P31107 (http://www.uniprot.org/uniprot/P31107.html) |
| | B3 | DRS-B3 | MAFLKKSVFLVLFLGLVSLSIC **EEEKREEENEEK-Q-EDDEQSEE *KR*** *ALWKNMLKGIGKLAGQAALGAVKTLVGAE* | | P81485 (http://www.uniprot.org/uniprot/P81485.html) |
| | B4 | DRS-B4 | MAFLKKSLFLVLFLGLVSLSIC **EEEKRENKDEIE-Q-EDDEQSEE *KR*** *ALWKDILKNVGKAAGKAVLNTVTDMVNQGEQ* | | P81486 (http://www.uniprot.org/uniprot/P81486.html) |
| | B6 | DRS-B6 | MAFLKKSLFLVLFLGLVSLSVC **EEEKRENEDEME-Q-EDDEQSEE *KR*** *ALWKDILKNAGKAALNEINQLVNQGEL* | | P81490 (http://www.uniprot.org/uniprot/P81490.html) |
| | B7 | DRS-B7 (DRG1) | MASLKKSLFLVLFLGLVSLSIC **EEEKRENEDEEE-Q-EDDEQSEM *KR*** *GLWSNIKTAGKEAAKAALKAAGKAALGAVTDAVGEQ* | | Q90ZK3 (http://www.uniprot.org/uniprot/Q90ZK3.html) |
| | B8 | DRS-B8 (DRG2) | MAFLKKSLFLVLFLGLVSLSIC **EEEKRENEDEEE-Q-EDDEQSEM *KR*** *GLWSKIKEAGKAALTAAGKAALGAVSDAVGEQ* | | Q90ZK5 (http://www.uniprot.org/uniprot/Q90ZK5.html) |
| | B9 | DRS-B9 (DRG3) | MAFLKKSLFLVLFLGLVSLSVC **EEEKRENEDEEE-Q-EDDEQSEE *KR*** *ALWKTIIKGAGKMIGSLAKNLLGSQAQPESEQ* | | P81488 (http://www.uniprot.org/uniprot/P81488.html) |
| | H1 | DRS-H1 (DShypo 01) | MAFLKKSLFLVLFLGMVSLSIC **EEEKRENEDEEL-Q-EDDEQSEM *KR*** *GLWSTIKNVGKEAAIAAGKAALGAL* | *P. hypochondrialis* | P84596 (http://www.uniprot.org/uniprot/P84596.html) |
| | H2 | DRS-H2 (DSN-1) | MDILKKSLFIVLFLGLVSLSIC **EEEKRENEDEEE-Q-EDDEQSEE *KR*** *GLWKSLLKNVGVAAGKAALNAVTDMVNQGEQ* | | Q0VZ37 (http://www.uniprot.org/uniprot/Q0VZ37.html) |
| | S1 | DRS-S1 (DS I) | MDILKKSLFLVLFLGLVSLSIC **EEEKRENEDEEK-Q-EDDEQSEM *KR*** *ALWKTMLKKLGTMALHAGKAALGAAADTISQGTQ* | | Q7T3K6 (http://www.uniprot.org/uniprot/Q7T3K6.html) |
| | S6 | DRS-S6 (SVI) | MDILKKSLFFILFLGLVSLSIS **EEEKRENEDEED-Q-EDDEQSEE *KR*** *GLWSKIKTAGKEAAKAAAKAAGKAALNAVSEAIGEQ* | | Q7T3K9 (http://www.uniprot.org/uniprot/Q7T3K9.html) |
| | S7 | DRS-S7 (SVII) | MDILKKSLFLVLFLGLISLSFC **EEEKRENEDEEE-Q-EDDEQSEE *KR*** *GLWKSLLKNVGKAAGKAALNAVTDMVNQGEQ* | | Q7T3K8 (http://www.uniprot.org/uniprot/Q7T3K8.html) |
| | S8 | DRS-S8 (SVIII) | MDILKKSLFLVLFLGLVSLSIC **EEEKRENEDEEK-Q-EDDEQSEM *KR*** *ALWKTMLKKLGTVALHAGKAALGAAADTISQGAQ* | *P. sauvagii* | Q7T3K7 (http://www.uniprot.org/uniprot/Q7T3K7.html) |
| | S9 | DRS-S9 (DSS9) | MAFLKKSLFLVLFLGLVSLSIC **DEEKRENEDEEN-Q-EDDEQSEM *RR*** *GLRSKIWLWVLLMIWQESNKFKKM* | | Q1EN15 (http://www.uniprot.org/uniprot/Q1EN15.html) |
| | S11 | DRS-S11 (DS11) | MAFLKKSLFLVLFLGMVSLSIC **EEEKRENEDEEE-Q-EDDEQSEE *KR*** *ALWKTLLKGAGKVFGHVAKQFLGSQGQPES* | | Q1EN13 (http://www.uniprot.org/uniprot/Q1EN13.html) |
| | S12 | DRS-S12 (DS12) | -ASLKKSLFLVLFLGLVSLSIC **EEEKRENEDEEN-Q-EDDEQSEM *RR*** *GLWSKIKEAAKTAGKMAMGFVNDMVGEQ* | | Q1EN12 (http://www.uniprot.org/uniprot/Q1EN12.html) |
| | S13 | DRS-S13 (DS13) | MAFLKKSLFLVLFLGLVSLSIC **DEEKRENEDEEN-Q-EDDEQSEM *RR*** *GLRSKIKEAAKTAGKMALGFVNDMAGEQ* | | Q1EN11 (http://www.uniprot.org/uniprot/Q1EN11.html) |
| Dermatoxin | B1 | DRT-B1 | MAFLKKSLFLVLFLGLVPLSLC **ESEKREGENEEE-Q-EDD-QSEE *KR*** *SLGSFLKGVGTTLASVGKVVSDQFGKLLQAGQG* | *P. bicolor* | Q9PT75 (http://www.uniprot.org/uniprot/Q9PT75.html) |
| | S1 | DRT-S1 | MAFLKKSLFLILFLGLVPLSFC **ENDKREGENEEE-Q-DDD-QSEE *KR*** *ALGTLLKGVGSAVATVGKMVADQFGKLLQAGQG* | *P. sauvagii* | Q5DVA5 (http://www.uniprot.org/uniprot/Q5DVA5.html) |
| Phylloseptin | AZ1 | PLS-AZ1 (PS-2) | LKKSLFLVVFLGLATLSIC **EEEKRETEEEEYNQGEDD-KSEE *KR*** *FLSLIPHAINAVSTLVHHFG* | | P85881 (http://www.uniprot.org/uniprot/P85881.html) |
| | AZ2 | PLS-AZ2 (PS-7) | MAFLKKSLFLVLFLGLVSLSIC **EEEKRETEEKENEQEDDD-KSEE *KR*** *FLSLIPHAINAVSAIAKHFG* | | P85882 (http://www.uniprot.org/uniprot/P85882.html) |
| | AZ3 | PLS-AZ3 (PS-8) | MAFLKKSLFLVLFLGLVSLSIC **EEEKRETEEEEYNQEDDD-KSEE *KR*** *FLSLIPTAINAVSALAKHFG* | *P. azurea* | P85883 (http://www.uniprot.org/uniprot/P85883.html) |
| | AZ4 | PLS-AZ4 (PS-12) | -------LVLFLGLVSLSIC **EEEKRETEEEENDQEEDD-KSEE *KR*** *FLSLLPSIVSGAVSLAKKLG* | | Q17UY9 (http://www.uniprot.org/uniprot/Q17UY9.html) |
| | AZ7 | PLS-AZ7 (PS-15) | ---LKKSLFLVLFLGLVSLSIC **EEEKRETEEKENEQ-EDD-KSEE *KR*** *LLSLVPHAINAVSAIAKHFG* | | Q0VKG9 (http://www.uniprot.org/uniprot/Q0VKG9.html) |
| | B1 | PLS-B1 (PBN1) | MAFLKKSLFLVLFLGLVSLSIC **EEEKRETEEKEYDQGEDD-KSEE *KR*** *FLSLIPHIVSGVAALAKHLG* | *P. bicolor* | Q800R3 (http://www.uniprot.org/uniprot/Q800R3.html) |
| | H2 | PLS-H2 (PS-2) | -AFLKKSLFLVLFLGLATLSIC **EEEKRETEEEEYNQEEDD-KSEE *KR*** *FLSLIPHAINAVSTLVHHFG* | | P84567 (http://www.uniprot.org/uniprot/P84567.html) |
| | H5 | PLS-H5 (PS-7) | MAFLKKSLFLVLFLGLVSLSIC **EEEKRETEEEENEQEDDD-KSEE *KR*** *FLSLIPHAINAVSAIAKHFG* | | P84572 (http://www.uniprot.org/uniprot/P84572.html) |
| | H6 | PLS-H6 (PS-8) | MAFLKKSLFLVLFLGLVSLSIC **EEEKRETEEEEYNQEDDD-KSEE *KR*** *FLSLIPTAINAVSALAKHFG* | *P. hypochondrialis* | Q0VZ41 (http://www.uniprot.org/uniprot/Q0VZ41.html) |
| | H7 | PLS-H7 (PS-9) | MAFLKKSLFLVLFLGLVSLSIC **EEEKRETEEEENDQEEDD-KSEE *KR*** *FLGLLPSIVSGAVSLVKKLG* | | Q0VZ38 (http://www.uniprot.org/uniprot/Q0VZ38.html) |
| | H8 | PLS-H8 (PS-10) | MAFLKKSLFLVLFLGLVSLSIC **EEEKRETEEEENDQEEDD-KSEE *KR*** *FLSLLPSLVSGAVSLVKKLG* | | Q0VZ39 (http://www.uniprot.org/uniprot/Q0VZ39.html) |
| | H9 | PLS-H9 (PS-11) | MAFLKKSLFLVLFLGLVSLSIC **EEEKRETEEEENDQEEDD-KSEE *KR*** *FLSLLPSLVSGAVSLVKILG* | | Q0VZ40 (http://www.uniprot.org/uniprot/Q0VZ40.html) |
| Phylloxin | B1 | PLX-B1 | MVFLKKSLLLVLFVGLVSLSIC **EENKREEHE-EIE-ENK-EKAEE *KR*** *GWMSKIASGIGTFLSGMQQG* | *P. bicolor* | P81565 (http://www.uniprot.org/uniprot/P81565.html) |
| | S1 | PLX-S1 | MVFLKKSLLLVLFVGLVSLSIC **EENKREEHE-EVE-ENA-EKAEE *KR*** *GWMSKIASGIGTFLSGVQQG* | *P. sauvagii* | Q5DVA6 (http://www.uniprot.org/uniprot/Q5DVA6.html) |
| Plasticin | B1 | PLC-B1 (PBN2) | MAFLKKSLFLVLFLALVPLSIC **EEKKSEEENEEK-Q-EDD-QSEE *KR*** *GLVTSLIKGAGKLLGGLFGSVTGGQS* | *P. bicolor* | Q800R4 (http://www.uniprot.org/uniprot/Q800R4.html) |
| | S1 | PLC-S1 (DS10) | MAFLKKSLFLVLFLALVPLSIC **EEEKREGENEKE-Q-EDDNQSEE *KR*** *GLVSDLLSTVTGLLGNLGGGGLKKI* | *P. sauvagii* | Q1EN14 (http://www.uniprot.org/uniprot/Q1EN14.html) |

[a] The peptides are named according to the nomenclature proposed by Amiche et al. (2008), the original nomenclature are in brackets

[b] The prepropeptide comprises a signal peptide (normal font) followed by an acidic propiece (bold font) that ends in a typical prohormone processing signal -KR- (italic bold font) that precedes the single downstream copy of the antimicrobial peptide progenitor sequence (italic font)

1994b). Furthermore, biophysical investigations indicate that the PM surface charge helps with the association of cationic peptides, but does not affect the channel structures themselves (Béven et al. 1999; Bechinger 2004; Gregory et al. 2008). Recent investigations by isothermal titration calorimetry (ITC) and by fluorescence spectroscopy support that the addition of cholesterol to phosphatidylcholine mimetic PMs lead to a decrease of dermaseptin membrane interactions and the concomitant disruption of the lipid bilayers (Verly et al. 2008). Other investigations through atomic force microscopy (AFM) indicated that dermaseptin is able to disrupt anionic PMs typical of bacteria (Silva et al. 2008). Fluorescence spectroscopy studies with liposomes and surface plasmonic resonance (SPR) analysis of the interaction of dermaseptins with immobilized bilayers demonstrated that the peptides preferentially bind to negatively charged membranes (Verly et al. 2009). Theoretical predictions and circular-dichroism (CD) studies indicated that dermaseptin is highly propense to fold into a cationic and amphipathic helix in hydrophobic medium, a structure indicative of PM lysing potential (Mor and Nicolas 1994a, b; Shalev et al. 2002; Lequin et al. 2003; Castiglione-Morelli et al. 2005). According to Verly et al. (2009), the unstabilization induced by the insertion of the peptide in one bilayer of the PM propagates from one bilayer to the next, triggering the loss of lipid order as a function of PM thickness (Verly et al. 2009). This effect is most pronounced on peptides that mismatch the bilayer thickness or those oriented parallel to the membrane surface (Harzer and Bechinger 2000).

The main advanced models for PM permeation by amphipathic helical peptides are very different, e.g., a canonical trans PM pore (barrel-stave); solubilization of the PM by a detergent-like action (Bechinger 2005) based on the amphipathic character of the AMPs and their massive accumulation into the PM (carpet model); and an intermediate two-state model (worm-hole) (Matsuzaki 1998; Papo and Shai 2003; Huang et al. 2004; Bechinger and Lohner 2006; Chan et al. 2006). The first two models are limited experimentally, e.g., the prediction that the PM permeation occurs at very low peptide:phospholipid ratio, assuming that peptide–peptide interaction is stronger than the peptide–phospholipid in the barrel-stave model, or the need for the whole covering of the organism by the peptide in the carpet model (Huang et al. 2004). The intermediate worm-hole or two-state model proposed independently by Matsuzaki and Huang (Matsuzaki 1998) tries to integrate three experimental observations: (1) the change in orientation undergone by a fraction of PM bound peptide once a threshold is reached, (2) peptide-induced phospholipid flip-flop, and (3) peptide translocation into the cytoplasm, ignored by the carpet and barrel-stave model (Rivas et al. 2009).

In this model, the massive union of the AMPs into the external monolayer of the PM leads to its expansion, causing a mechanical stress. Once a threshold is reached, a fraction of the peptides lying parallel to the plane of the PM, change their orientation from parallel to transversal, promoting a positive curvature of the PM and forming a mixed phospholipid–peptide toroidal pore, where the hydrophobic lining is provided both by the polar heads of the phospholipids and the hydrophilic face of the peptides. This pore also acts as catalyst in the phospholipid interchange between the two leaflets. This pore is transitory and, when it disappears, stochastically sends it's forming monomers to either side of the PM (Rivas et al. 2009). This more comprehensive model is a subtle tour de force refinement over the detergent carpet-like model and supports the step-wise increase in conductivity observed for several AMPs (Rivas et al. 2009).

Two other models are also proposed; a fourth mechanism, the "aggregate model" (Chan et al. 2006), relaxes the structural requirements intrinsic to the toroidal model, mostly applicable to α-helical peptides, to accommodate peptides not adopting this prototypical cylindrical shape (Rivas et al. 2009) and a fifth model, the so called "Droste mechanism" (Sengupta et al. 2008), indicating that the toroidal lumen adopts a poor orientation and the hydrophilic lining is mostly provided by the positive curvature of the phospholipids, with a scarce role of the peptide, which accumulates at the rim of the pore and stabilizes it (Rivas et al. 2009).

## Dermaseptin

The dermaseptin family comprises a large class of PM damaging polycationic (K-rich) peptides with different lengths (28–34 residues) and amino acid sequences that undergo coil-to-helix transition upon binding to lipid bilayers (Nicolas and El Amri 2009) found in the skin of *Phyllomedusa azurea*, *P. bicolor*, *P. burmeisteri*, *P. distincta*, *P. hypochondrialis*, *P. oreades*, *P. rohdei*, *P. sauvagii*, *P. tarsius*, *P. tomopterna*, and *P. trinitatis* (see Table 2). Generally, they all have conserved a W residue at position 3 usually preceded by AL- or GL-, an AG[A]K[Q]A[M]A[V]L[G]G[N/K]A[F]V[A/L] consensus motif in the middle or C-terminal region and positive charge due to the presence of K residues that punctuate an alternating hydrophobic and hydrophilic sequence (Table 4).

The first dermaseptin described was a 34-amino acid antimicrobial peptide termed dermaseptin-S1 identified in skin extract of *P. sauvagii* (Mor et al. 1991a). This peptide has lytic activity against Gram-positive and Gram-negative bacteria, yeast, and protozoa, but does not damage mammalian cells. This was the first gene-encoded eukaryotic peptide with lethal effect against filamentous fungi

responsible for opportunistic infections in immunodeficiency syndrome or immunosuppressed individuals (Nicolas and El Amri 2009). This was followed by the isolation of adenoregulin (also named dermaseptin-B2) from *P. bicolor* skin, a peptide that interacts with the adenosine receptor (Daly et al. 1992), and dermaseptin-B1 (Mor et al. 1994a, c). These two peptides were thought to be unrelated, but the cloning of their precursor polypeptides revealed the existence of the canonical precursor (Amiche et al. 1993; Vouille et al. 1997). After that, additional members of the dermaseptin family were rapidly identified in various South American anuran species (Lequin et al. 2006; Nicolas and El Amri 2009).

Dermaseptins and their analogs have lytic activity in vitro against a broad spectrum of free-living microorganism strains of the wall-less bacteria: *Acholeplasma laidlawii*, *Spiroplasma apis*, *S. citri*, *S. floricola*, and *S. melliferum* (Fleury et al. 1998); Gram-negative bacteria: *Aerornonas caviae* (Mor and Nicolas 1994a, b; Strahilevitz et al. 1994), *Acholeplasma laidlawii* (Fleury et al. 1998), *Acetobacter calcoaceticus* (Brand et al. 2002), *Escherichia coli* (Mor and Nicolas 1994a, b; Strahilevitz et al. 1994; Fleury et al. 1998; Batista et al. 1999; Silva et al. 2000; Brand et al. 2002; Conceição et al. 2006; Brand et al. 2006b; Leite et al. 2008), *Neisseria gonorrhoeae* (Rydlo et al. 2006; Zairi et al. 2009), and *Pseudomonas aeruginosa* (Fleury et al. 1998; Batista et al. 1999; Silva et al. 2000; Brand et al. 2002, 2006b; Conceição et al. 2006; Leite et al. 2008); Gram-positive bacteria: *Corynebacterium glutamicum* (Fleury et al. 1998), *Enterococcus faecalis* (Batista et al. 1999; Silva et al. 2000), *Micrococcus luteus* (Conceição et al. 2006), *Nocardia* spp. (Leite et al. 2008), *Nocardia brasiliensis* (Mor and Nicolas 1994a, b; Strahilevitz et al. 1994), *Staphylococcus aureus* (Strahilevitz et al. 1994; Fleury et al. 1998; Batista et al. 1999; Silva et al. 2000; Brand et al. 2002, 2006b; Conceição et al., 2006; Leite et al. 2008), *Streptococcus dysgalactiae* (Leite et al. 2008), and *S. uberis* (Leite et al. 2008); the fungi: *Aspergillus fumigatus* (Mor and Nicolas 1994a, b), *Arthroderma simii*, *Cryptococcus neofonnans* (Strahilevitz et al. 1994; Mor and Nicolas 1994a, b), *Candida albicans* (Mor and Nicolas 1994a, b; Strahilevitz et al. 1994; Leite et al. 2008; Zairi et al. 2008), *C. tropicalis*, *C. guilliermondii* (Leite et al. 2008), *Microsporum canis*, *Tricophyton rubrum* (Strahilevitz et al. 1994; Mor and Nicolas 1994a, b); Protozoa: *Leishmania major* (promastigotes) (Feder et al. 2000; Kustanovich et al. 2002; Gaidukov et al. 2003), *L. mexicana* (promastigotes) (Hernandez et al. 1992; Mor and Nicolas 1994b), *L. amazonensis* (epimastigotes and promastigotes) (Brand et al. 2006b), *L. chagasi* (promastigotes) (Zampa et al. 2009), *Plasmodium falciparum* (trophozoites) (Ghosh et al. 1997; Krugliak et al. 2000; Dagan et al. 2002), and *Trypanosoma cruzi* (trypomastigotes) (Brand et al. 2002); and Virus: HSV-1 (Belaid et al. 2002) and HIV-1 (Lorin et al. 2005; Zairi et al. 2009). The wall-less bacteria, *Mycoplasma gallisepticum* and *M. mycoides*, and Gram-negative bacteria, *Salmonella typhimurium*, are resistant to dermaseptin B9 (DRG3) from *P. bicolor* (Fleury et al. 1998).

Despite the sequence similarities, the dermaseptins differ in their action efficiency (Nicolas and El Amri 2009; Rivas et al. 2009). But they present rapid and irreversible antimicrobial effect and no toxic effects in mammalian cells in vitro (Kustanovich et al. 2002; Navon-Venezia et al. 2002). However, dermaseptin-S4 analogs had a potent activity against human sperm (Zairi et al. 2009).

Some of the peptides from the dermaseptin superfamily present other additional biological functions that have unclear relations with pathogen clearance, e.g., dermaseptin B2 (adenoregulin): isolated by Daly et al. (1992) as a peptide that stimulated the binding of agonists to A1 adenosine receptors and also enhanced the binding of agonists to several G-protein coupled receptors in rat brain PMs through a mechanism involving enhancement of guanyl nucleotide exchange at G-proteins (Shin et al. 1994); dermaseptin-B4: stimulates insulin release by acute incubation with glucose-responsive cells (Marenah et al. 2004); dermaseptin-S1: stimulates the production of reactive oxygen species and release of myeloperoxidase by polymorphonuclear leukocytes (Ammar et al. 1998).

## Dermatoxin

Two dermatoxins were identified in the skin secretions of *Phyllomedusa bicolor*, *P. sauvagii*, and *P. tarsius*. The primary structures of dermatoxin are highly conserved exhibiting few chemically conservative amino acid substitutions (Chen et al. 2005a). In contrast to dermaseptins, dermatoxins do not have W in its composition, having instead a G residue at position 3, or an R residue preceded by AL- or SL- and followed by a conserved KGVG consensus sequence. From the middle of the C-terminus region, a high conserved sequence AT[G/S]VGKV[M/I]VA [S]DQFG[D]KLLQ[E]A is observed (Table 5).

Another interesting feature was the presence of the C-terminal dipeptide -GQ on the *P. bicolor* dermatoxin-B1 (DRT-B1) (Amiche et al. 2000; Chen et al. 2005a). The dermatoxin is structured as a preproprotein of the dermaseptin family of peptide precursors (Table 3) and characterized by strongly conserved preproregions followed by C-terminal sequence domain of precursors of the dermaseptin family (Amiche et al. 2000).

Dermatoxin presents a cationic amphipathic α-helical conformation in low polarity media, which mimics the lipophilicity of the PM of target microorganisms (Amiche et al. 2000; Chen et al. 2005a). It is membranotropic and antimicrobial with a sequence and cell killing mechanism diverse from dermaseptin and phylloxin (Amiche et al.

**Table 4** Primary structures of dermaseptins from *Phyllomedusa* species

| Species | Dermaseptin | Abbreviation | Peptide | Digital abstract |
|---|---|---|---|---|
| *P. azurea* | AZ2 | DRS-AZ2 (DMS2)[a] | GLWSKIKDVAAA**AGKAALGAV**NEALGEQ | P84937 (http://www.uniprot.org/uniprot/P84937.html) |
| | AZ3 | DRS-AZ3 (DMS3)[a] | GLWSTIKNVAAA**AGKAALGAL**–$NH_2^b$ | Q17UY8 (http://www.uniprot.org/uniprot/Q17UY8.html) |
| | AZ4 | DRS-AZ4 (DMS4)[a] | GLWSTIKNVGKEAAIA**AGKAALGAL**–$NH_2$ | Q1EJP4 (http://www.uniprot.org/uniprot/Q1EJP4.html) |
| | AZ5 | DRS-AZ5 (DMS5)[a] | GLWSTIKNVGKEAAIA**AGKAVLGS****L**–$NH_2$ | Q1EJP5 (http://www.uniprot.org/uniprot/Q1EJP5.html) |
| | AZ6 | DRS-AZ6 (DMS6)[a] | GLWSTIKQKGKEAAIA**AAKAAG**Q**AA**LGAL | P84936 (http://www.uniprot.org/uniprot/P84936.html) |
| *P. bicolor* | B1 | DRS-B1 (B1) | AMWKDVLKKIGTVALH**AGKAALGAV**ADTISQ-$NH_2^b$ | P80282 (http://www.uniprot.org/uniprot/P80282.html) |
| | B2 | DRS-B2 (B2) | GLWSKIKEVGKEAAKA**AAKAAGKAA**LGAVSEAV-$NH_2^b$ | P31107 (http://www.uniprot.org/uniprot/P31107.html) |
| | B3 | DRS-B3 (B3) | ALWKNMLKGIGKL**AGQAALGAV**KTLVGAE | P81485 (http://www.uniprot.org/uniprot/P81485.html) |
| | B4 | DRS-B4 (B4) | ALWKDILKNVGKA**AGKA**V**LN**T**V**TDMVNQ-$NH_2$ | P81486 (http://www.uniprot.org/uniprot/P81486.html) |
| | B5 | DRS-B5 (B5) | GLWNKIKEAASKA**AGKAALGFV**NEMV | P81487 (http://www.uniprot.org/uniprot/P81487.html) |
| | B6 | DRS-B6 (B6) | ALWKDILKN**AGKAALN**EINQLVNQ-$NH_2$ | P81490 (http://www.uniprot.org/uniprot/P81490.html) |
| | B7 | DRS-B7 (DRG1) | GLWSNIKTAGKEAAKAALKA**AGKAALGAV**TDAV-$NH_2^b$ | Q90ZK3 (http://www.uniprot.org/uniprot/Q90ZK3.html) |
| | B8 | DRS-B8 (DRG2) | GLWSKIKEAGKAALTA**AGKAALGAV**SDAV-$NH_2^b$ | Q90ZK5 (http://www.uniprot.org/uniprot/Q90ZK5.html) |
| | B9 | DRS-B9 (DRG3) | ALWKTIIKG**AGKM**I**G**S**L**AKNLLGSQAQPES | P81488 (http://www.uniprot.org/uniprot/P81488.html) |
| *P. burmeisteri* | BU1 | DRS-BU1 (DS III-like) | ALWKNMLKGIGKL**AGKAALGAV**K | P86281 (http://www.uniprot.org/uniprot/P86281.html) |
| | BU2 | DRS-BU2 (DRS-DI4-like) | ALWKNMLKGIGKL**AGQAALGAV**KTLVGA | P86279 (http://www.uniprot.org/uniprot/P86279.html) |
| | BU3 | DRS-BU3 (DS VIII-like) | ALWKTMLKKLGTVALH**AGKAALGAA**ADTISQGA | P86280 (http://www.uniprot.org/uniprot/P86280.html) |
| *P. distincta* | DI1 | DRS-DI1 (DD K) | GLWSKIKAAGKEAAKAAAKA**AGKAALNAV**SEAV | P83638 (http://www.uniprot.org/uniprot/P83638.html) |
| | DI2 | DRS-DI2 (DD L) | ALWKTLLKNVGKA**AGKAALNAV**TDMVNQ | P83639 (http://www.uniprot.org/uniprot/P83639.html) |
| | DI3 | DRS-DI3 (DD M) | ALWKTMLKKLGTMALH**AGKAAFGAA**ADTISQ | P83640 (http://www.uniprot.org/uniprot/P83640.html) |
| | DI4 | DRS-DI4 (DD Q1) | ALWKNMLKGIGKL**AGQAALGAV**KTLVGAES | P83641 (http://www.uniprot.org/uniprot/P83641.html) |
| | D15 | DRS-DI5 (DD Q2) | GLWSKIKE**AAK**T**AG**LM**A**MGFVNDMV | P83642 (http://www.uniprot.org/uniprot/P83642.html) |
| *P. hypochondrialis* | H1 | DRS-H1 (DShypo 01)[a] | GLWSTIKNVGKEAAIA**AGKAALGAL**-$NH_2$ | P84596 (http://www.uniprot.org/uniprot/P84596.html) |
| | H2 | DRS-H2 (DShypo 02)[a] | GLWKSLLKNVGVA**AGKAALNAV**TDMVNQ | P84597 (http://www.uniprot.org/uniprot/P84597.html) |
| | H3 | DRS-H3 (DShypo 03)[a] | ALWKDVLKKIGTVALH**AGKAAFGAA**ADTISQGGS | P84598 (http://www.uniprot.org/uniprot/P84598.html) |
| | H4 | DRS-H4 (DShypo 04)[a] | GLWSTIKQKGKEAAIA**AAKAAGKAV**LNAASEAL-$NH_2$ | P84599 (http://www.uniprot.org/uniprot/P84599.html) |
| | H5 | DRS-H5 (DShypo 05)[a] | GLWSTIKQKGKEAAIA**AAKAAGQAA**LGAL-$NH_2$ | P84600 (http://www.uniprot.org/uniprot/P84600.html) |
| | H6 | DRS-H6 (DShypo 06)[a] | GLWSTIKQKGKEAAIA**AAKAAGQAV**LNSASEAL-$NH_2$ | P84601 (http://www.uniprot.org/uniprot/P84601.html) |
| | H7 | DRS-H7 (DShypo 07)[a] | GLWSTIKQKGKEAAIA**AAKAAGQAA**LNAASEAL-$NH_2$ | P84880 (http://www.uniprot.org/uniprot/P84880.html) |
| | H8 | DRS-H8 (DSN-2)[a] | ALWKSLLKNVGVA**AGKAALNAV**TDMVNQ | Q0VZ36 (http://www.uniprot.org/uniprot/Q0VZ36.html) |
| *P. oreades* | O1 | DRS-O1 (DS01) | GLWSTIKQKGKEAAIA**AAKAAGQAA**LGAL-$NH_2$ | P83637 (http://www.uniprot.org/uniprot/P83637.html) |
| *P. sauvagii* | S1 | DRS-S1 (DS I) | ALWKTMLKKLGTMALH**AGKAALGAA**ADTISQGTQ | P24302 (http://www.uniprot.org/uniprot/P24302.html) |
| | S2 | DRS-S2 (DS II) | ALWFTMLKKLGTMALH**AGKAALGAA**ANTISQGTQ | P80278 (http://www.uniprot.org/uniprot/P80278.html) |
| | S3 | DRS-S3 (DS III) | ALWKNMLKGIGKL**AGKAALGAV**KKLVGAES | P80279 (http://www.uniprot.org/uniprot/P80279.html) |
| | S4 | DRS-S4 (DS IV) | ALWMTLLKKVLKA**AAKALNAV**LVGANA | P80280 (http://www.uniprot.org/uniprot/P80280.html) |
| | S5 | DRS-S5 (DS V) | GLWSKIKTAGKSVAKA**AAKAAVKAV**TNAV | P80281 (http://www.uniprot.org/uniprot/P80281.html) |
| | S6 | DRS-S6 (DS VI) | GLWSKIKTAGKEAAKA**AAKAAGKAA**LNAVSEAI | Q7T3K9 (http://www.uniprot.org/uniprot/Q7T3K9.html) |
| | S7 | DRS-S7 (DS VII) | GLWKSLLKNVGKA**AGKAALNAV**TDMVNQ | Q7T3K8 (http://www.uniprot.org/uniprot/Q7T3K8.html) |
| | S8 | DRS-S8 (DS VIII) | ALWKTMLKKLGTVALH**AGKAALGAA**ADTISQ | Q7T3K7 (http://www.uniprot.org/uniprot/Q7T3K7.html) |
| | S9 | DRS-S9 (S9) | GLRSKIWLWVLLMIW**Q**ESN**KF**KKM | Q1EN15 (http://www.uniprot.org/uniprot/Q1EN15.html) |
| | S11 | DRS-S11 (S11) | ALWKTLLKG**AGK**VF**G**HV**A**KQFLGSQGQPES | Q1EN13 (http://www.uniprot.org/uniprot/Q1EN13.html) |
| | S12 | DRS-S12 (S12) | GLWSKIKEAAKT**AGKMA**M**GFV**NDMVGEQ | Q1EN12 (http://www.uniprot.org/uniprot/Q1EN12.html) |
| | S13 | DRS-S13 (S13) | GLRSKIKEAAKT**AGKMALGFV**NDMAGEQ | Q1EN11 (http://www.uniprot.org/uniprot/Q1EN11.html) |

**Table 4** continued

| Species | Dermaseptin | Abbreviation | Peptide | Digital abstract |
|---|---|---|---|---|
| *P. tarsius* | T1 | DRS-T1 (DStar 01) | GLWSKIKETGKE**AAKAAGKAA**LNKIAEAV-$NH_2$ | P84921 (http://www.uniprot.org/uniprot/P84921.html) |
| | T2 | DRS-T2 (DStar 02) | ALWKDILKNV**GKAAGKAV**LNTVTDMVNQ-$NH_2$ | P84922 (http://www.uniprot.org/uniprot/P84922.html) |
| | T3 | DRS-T3 (DStar 03) | GLFKTLIKG**AGKM**L**G**HV**A**KQFLGSQGQPES | P84923 (http://www.uniprot.org/uniprot/P84923.html) |
| | T4 | DRS-T4 (DStar 04) | ALWKDILKN**AGKAALN**EINQIVQ-$NH_2$ | P84924 (http://www.uniprot.org/uniprot/P84924.html) |
| | T5 | DRS-T5 (DStar 05) | GLWSKIKEAAKT**AGKAAMGFV**NEMV-$NH_2$ | P84925 (http://www.uniprot.org/uniprot/P84925.html) |
| | T6 | DRS-T6 (DStar 06) | ALWKNMLKGIGKL**AGQAALGAV**KTLVGA | P84926 (http://www.uniprot.org/uniprot/P84926.html) |
| | T7 | DRS-T7 (DStar 07) | ALWKDVLKKIGTVALH**AGKAALGAV**ADTISQ-$NH_2$ | P84927 (http://www.uniprot.org/uniprot/P84927.html) |

The bold residues belong to the consensus motif. Names used are in accordance with the nomenclature proposed by Amiche et al. (2008). Abbreviations used before the new nomenclature are in brackets

[a] New names proposed for peptides isolated from *P. azurea* and *P. hypochondrialis* according to the nomenclature rules proposed by Amiche et al. (2008). Before *P. azurea* was renamed by Caramaschi (2006) the species used to be named as *P. hypochondrialis azurea* (Calderon et al. 2009b)

[b] The C-terminal amidation given is based on similarity and not on experimental findings

2000). Observation of bacterial cells by reflected light fluorescence microscopy after DNA-staining supports the cell-killing mechanism based upon the alteration of PM permeability rather than PM solubilization, possibly related to ion-conducting channels through the PM (Amiche et al. 2000).

The antimicrobial activity spectrum of dermatoxin includes stains of wall-less and Gram-positive bacteria, and also, though to a lesser extent, Gram-negative bacteria. The wall-less bacteria: *Acholeplasma laidlawii*, *Spiroplasma melliferum*; Gram-negative bacteria: *Sinorhizobium meliloti*; and Gram-positive bacteria: *Bacillus megaterium* and *Corynebacterium glutamicum* are susceptible to DRT-B1. The Gram-negative bacteria: *Burkholderia cepacia*, *Pseudomonas aeruginosa* and *Salmonella typhimurium*; and Gram-positive bacteria: *Staphylococcus aureus* are resistant to DRT-B1 (Amiche et al. 2000). Amiche et al. (2000) argue that the higher resistance against dermatoxin by Gram-negative bacteria might be related to the presence of a second PM in their envelope.

## Distinctin

Distinctin, a prototypical member of a family of antimicrobial peptides, is a 5.4-kDa heterodimeric antimicrobial peptide from *Phyllomedusa distincta* with two linear peptide chains of 22 and 25 amino acid residues joined by a single intermolecular disulfide bond (Fig. 2) (Batista et al. 2001). To date, only the peptide that shows sequence similarity to the distinctin chain B was the distinctin-like peptide (ppdis-H1) from *P. azurea*. They have in common a high conserved N-terminal sequence NLVSG[A]-LIEA[G]RKYL (Table 6). Heterodimeric structures joined by a single intermolecular S–S bond were reported in invertebrates' neurotoxins inhibiting neurotransmitter release, imperatoxin I, and L-bungarotoxins (Kwong et al. 1995). CD and FTIR studies indicate that this molecule adopts, in aqueous solution, a structure with a significant percentage of antiparallel $\beta$-sheet (Batista et al. 2001) whereas the CD and FTIR spectroscopy experimental data indicate that the distinctin heterodimer adopts helical conformations with a lower $\beta$-sheet content in PM environments (Serra et al. 2008). NMR experiments indicated that the peptide helices are oriented almost parallel to the PM surface, thereby reflecting the amphipathic distribution of apolar and hydrophilic amino acid side chains (Bechinger 1999; Bechinger et al. 2001; Resende et al. 2008). According to Serra et al. (2008), the experimental output recorded so far for the distinctin mechanism of insertion into PMs is compatible with a barrel-stave pore (Serra et al. 2008).

The antimicrobial activity spectrum of distinctin includes strains of the Gram-negative bacteria: *Acinetobacter baumannii*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Serratia marcescens*, and *Stenotrophomonas maltophilia*; and Gram-positive bacteria: *Enterococcus faecalis*, *E. faecium*, *Staphylococcus aureus*, and *Streptococcus pneumoniae* (Batista et al. 2001; Giacometti et al. 2006; Serra et al. 2008).

## Phylloseptin

Phylloseptins are AMPs of 19–21 residues (1.7–2.1 kDa) found in the skin secretions of the *Phyllomedusa azurea*, *P. bicolor*, *P. burmeinsteri*, *P. hypochondrialis*, *P. oreades*, *P. rohdei*, *P. tarsius*, and *P. tomopterna* (see Table 2). Their common structural features include a highly conserved sequence FLSLI[L]P in the N-terminal region and

C-terminal amidation (Leite et al. 2005) (see Table 7). Phylloseptins exemplify that considerable differences in biological activity may rely upon minor modifications of the primary sequence of model compounds, even when overall amino acid composition is kept constant (Wieprecht et al. 1997). Phylloseptin peptides adopt α-helical conformations in PM environments stabilized by electrostatic interactions of the helix dipole and others, such as hydrophobic and capping interactions (Resende et al. 2008). AFM experiments indicated that the bacteriolytic properties of these peptides might be related to their disruptive action on the PM characterized by a number of bubble-like formations, preceding every cell lysis (Leite et al. 2005).

The antimicrobial activity spectrum of phylloseptins includes strains of the Gram-negative bacteria: *Acinetobacter calcoaceticus*, *Escherichia coli*, and *Pseudomonas aeruginosa* (Leite et al. 2005; Resende et al. 2008); Gram-positive bacteria: *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Streptococcus agalactiae*; Fungi: *Candida albicans* (Resende et al. 2008); and Protozoa: *Leishmania amazonensis* (promastigotes) (Kückelhaus et al. 2009), *Plasmodium falciparum* (rings, trophozoites, and schizonts) (Kückelhaus et al. 2009), and *Trypanosoma cruzi* (trypomastigotes) (Leite et al. 2005).

Besides, this peptide exhibited negligible effects on red blood cells (Leite et al. 2005) and some toxic effect to mammalian cells only at very high concentrations (Kückelhaus et al. 2006, 2009).

## Phylloxin

Phylloxin is a family of cationic and amphipathic peptides that have very similar N-terminal preprosequences followed by marked C-terminal domains. Two phylloxins, 19 residues long from *Phyllomedusa* skin, were identified. Phylloxin B1 from *P. bicolor* (PLX-B1) (Pierre et al. 2000; Chen et al. 2005b) and phylloxin S1 from *P. sauvagii* (PLX-S1) (Chen et al. 2005a) (Table 8). The primary structures of the two phylloxins are extremely conserved, exhibiting only one conservative amino acid substitution at position 17, containing M or V for PLX-B1 and PLX-S1, respectively.

Phylloxins are members of the family of preprodermorphin/dermaseptins-derived peptides (see Table 3). Despite the considerable similarity between the phylloxin polypeptide precursor and the preprodermaseptin-B1 (Pierre et al. 2000), there is no homology between the phylloxin and dermaseptins (Pierre et al. 2000), in spite of some resemblance to the levitide-precursor fragment and the xenopsin-precursor fragment, two AMPs isolated from *Xenopus laevis* (Poulter et al. 1988; Fleury et al. 1996; Pierre et al. 2000).

The preprophylloxin consists of a putative signal peptide of 20–23 residues, a typical propeptide convertase processing site (–KR–), an intervening acidic amino acid residue-rich spacer peptide, a second typical processing site and a terminal antimicrobial peptide-encoding domain (the hypervariable domain) (Chen et al. 2005a).

Circular dichroism (CD) spectra of phylloxin in low polarity medium, mimicking the lipophilicity of the PM of target microorganisms, indicated 60–70% α-helical conformation, and predictions of secondary structure suggested that the peptide can be configured as an amphipathic helix spanning residues 1–19 (Pierre et al. 2000).

The antimicrobial activity spectrum of phylloxin includes the strains of the wall-less bacteria: *Acholeplasma laidlawii* and *Spiroplasma melliferum*; Gram-negative bacteria: *Escherichia coli*; and Gram-positive bacteria:

**Table 5** Primary structures of dermatoxins from *Phyllomedusa* species

| Species | Dermatoxin[a] | Abbreviation[a] | Sequence | Digital abstract |
|---|---|---|---|---|
| *P. bicolor* | B1 | DRT-B1 | S**L**GSF**LKGVG**TTL**A**S**VGK**V**V**S**DQF**G**K**LLQAGQG** | Q9PT75 (http://www.uniprot.org/uniprot/Q9PT75.html) |
| *P. sauvagii* | S1 | DRT-S1 | A**L**GTL**LKGVG**SAV**A**T**VGK**M**V**A**DQF**G**K**LLQAGQG** | Q5DVA5 (http://www.uniprot.org/uniprot/Q5DVA5.html) |
| *P. tarsius* | T1 | DRT-T1 (DStar 08) | S**L**RGF**LKGVG**TAL**A**G**VGK**V**V**A**DQF**D**K**LLQAGQ**-$NH_2$ | P84928 (http://www.uniprot.org/uniprot/P84928.html) |

The bold residues are conserved

[a] The peptides are named according to the nomenclature proposed by Amiche et al. (2008), the original nomenclature are in brackets

**Table 6** Primary structures of distinctin and distinctin-like peptide from *P. azurea*

| Species | Name | Sequence | Digital abstract |
|---|---|---|---|
| *P. distincta* | Distinctin Chain B | **NLVSGLIEARKYL**EQLHR**KL**KNC**K**V | – |
| *P. azurea* | Distinctin-like (ppdis-H1) | **NLVSALIEGRKYL**KNVLK**KL**NRL**K**EKNKAKNSKENN | Q17UZ0 (http://www.uniprot.org/uniprot/Q17UZ0.html) |

The bold residues are conserved

*Micrococcus luteus*. The Gram-negative bacteria: *Pseudomonas aeruginosa*, *Rhizobium meliloti*, and *Salmonella typhimuriu*; and Gram-positive bacteria: *Corynebacterium glutamicum* and *Staphylococcus aureus* show resistance to phylloxin B1 (Pierre et al. 2000).

## Plasticin

Plasticins are 23 long-residue GL-rich dermaseptin-related peptides with very similar amino acid sequences, hydrophobicities, and amphipathicities, but differ in their PM damaging properties and structuration (i.e., destabilized helix states, $\beta$-hairpin, $\beta$-sheet, and disordered states) at anionic and zwitterionic PM interfaces (El Amri et al. 2006). To date, two plasticins were described in *Phyllomedusa* secretions (Table 9): the cationic peptide plasticin-B1 (PTC-B1) from *P. bicolor*, which was previously described with the name PBN2 (Vanhoye et al. 2004), and plasticin-S1 (PTC-S1) from *P. sauvagii*, which was previously described as dermaseptin-S10 (Amiche et al. 2008).

```
     ENREVPPGFTALIKTLRKCKII  (chain 1)
                            |
NLVSGLIEARKYLEQLHRKLKNCKV   (chain 2)
```

**Fig. 2** Primary structure of distinctin from *P. distincta*

Structural malleability of plasticins in aqueous solutions and parameters governing their ability to fold within $\beta$-hairpin-like structures were analyzed through CD and FTIR spectroscopic studies completed by molecular dynamics simulations in polar mimetic media (El Amri et al. 2006).

All plasticins present a turn region that does not always result in folding into a $\beta$-hairpin-shaped conformation. Residue at position 8 plays a major role in initiating the folding, while position 12 is not critical (Bruston et al. 2007). Conformational stability has no major impact on antimicrobial efficacy (Bruston et al. 2007). However, preformed $\beta$-hairpin in solution may act as a conformational lock that prevents the switch to $\alpha$-helical structure (Bruston et al. 2007). This lock lowers the antimicrobial efficiency and explains subtle differences in potencies of the most active antimicrobial plasticins (Bruston et al. 2007).

**Table 7** Primary structures of phylloseptins from *Phyllomedusa* species

| Species | Phylloseptin[a] | Abbreviation[a] | Peptide | Digital abstract |
|---|---|---|---|---|
| *P. azurea* | AZ1 | PLS-AZ1 (PS-2) | **FLSLIP**HAINAVSTLVHHF-$NH_2$ | P85881 (http://www.uniprot.org/uniprot/P85881.html) |
| | AZ2 | PLS-AZ2 (PS-7) | **FLSLIP**HAINAVSAIAKHF-$NH_2$ | P85882 (http://www.uniprot.org/uniprot/P85882.html) |
| | AZ3 | PLS-AZ3 (PS-8) | **FLSLLP**TAINAVSALAKHF-$NH_2$ | P85883 (http://www.uniprot.org/uniprot/P85883.html) |
| | AZ4 | PLS-AZ4 (PS-12) | **FLSLLP**SIVSGAVSLAKKL-$NH_2$ | Q17UY9 (http://www.uniprot.org/uniprot/Q17UY9.html) |
| | AZ5 | PLS-AZ5 (PS-13) | **FLSLIP**HAINAVGVHAKHF-$NH_2$ | P84938 (http://www.uniprot.org/uniprot/P84938.html) |
| | AZ6 | PLS-AZ6 (PS-14) | **FLSLIP**AAISAVSALADHF-$NH_2$ | P84939 (http://www.uniprot.org/uniprot/P84939.html) |
| | AZ7 | PLS-AZ7 (PS-15) | L**LSLVP**HAINAVSAIAKHF-$NH_2$ | Q0VKG9 (http://www.uniprot.org/uniprot/Q0VKG9.html) |
| *P. bicolor* | B1 | PLS-B1 (PBN-1) | **FLSLIP**HIVSGVAALAKHL-$NH_2$ | Q800R3 (http://www.uniprot.org/uniprot/Q800R3.html) |
| *P. burmeisteri* | BU1 | PLS-BU1 (Bu-1) | **FL**ISI**P**YSASIGGTATLTGTA-$NH_2$ | P86282 (http://www.uniprot.org/uniprot/P86282.html) |
| | BU2 | PLS-BU2 (Bu-2) | **FL**LSL**P**HLASGLASLVLSK-$NH_2$ | P86283 (http://www.uniprot.org/uniprot/P86283.html) |
| *P. hypochondrialis* | H1 | PLS-H1 (PS-1) | **FLSLIP**HAINAVSAIAKHN-$NH_2$ | P84566 (http://www.uniprot.org/uniprot/P84566.html) |
| | H2 | PLS-H2 (PS-2) | **FLSLIP**HAINAVSTLVHHF-$NH_2$ | P84567 (http://www.uniprot.org/uniprot/P84567.html) |
| | H3 | PLS-H3 (PS-3) | **FLSLIP**HAINAVSALANHG-$NH_2$ | P84568 (http://www.uniprot.org/uniprot/P84568.html) |
| | H4 | PLS-H4 (PS-6) | --**SLIP**HAINAVSAIAKHF-$NH_2$ | P84571 (http://www.uniprot.org/uniprot/P84571.html) |
| | H5 | PLS-H5 (PS-7) | **FLSLIP**HAINAVSAIAKHF-$NH_2$ | P84572 (http://www.uniprot.org/uniprot/P84572.html) |
| | H6 | PLS-H5 (PS-8) | **FLSLLP**TAINAVSALAKHF-$NH_2$ | Q0VZ41 (http://www.uniprot.org/uniprot/Q0VZ41.html) |
| | H7 | PLS-H6 (PS-9) | **FL**G**LLP**SIVSGAVSLVKKLG-$NH_2$ | Q0VZ38 (http://www.uniprot.org/uniprot/Q0VZ38.html) |
| | H8 | PLS-H7 (PS-10) | **FLSLLP**SLVSGAVSLVKKL-$NH_2$ | Q0VZ39 (http://www.uniprot.org/uniprot/Q0VZ39.html) |
| | H9 | PLS-H8 (PS-11) | **FLSLLP**SLVSGAVSLVKIL-$NH_2$ | Q0VZ40 (http://www.uniprot.org/uniprot/Q0VZ40.html) |
| *P. oreades* | O1 | PLS-O1 (PS-4) | **FLSLIP**HAINAVSTLVHHSG-$NH_2$ | P84569 (http://www.uniprot.org/uniprot/P84569.html) |
| | O2 | PLS-O2 (PS-5) | **FLSLIP**HAINAVSAIAKHS-$NH_2$ | P84570 (http://www.uniprot.org/uniprot/P84570.html) |
| *P. tarsius* | T1 | PLS-T1 (PStar 01) | **FLSLIP**KIAGGIASLVKNL-$NH_2$ | P84929 (http://www.uniprot.org/uniprot/P84929.html) |
| | T2 | PLS-T2 (PStar 02) | **FLSLIP**HIATGIAALAKHL-$NH_2$ | P84930 (http://www.uniprot.org/uniprot/P84930.html) |
| | T3 | PLS-T3 (PStar 03) | **FFSMIP**KIATGIASLVKNL-$NH_2$ | P84931 (http://www.uniprot.org/uniprot/P84931.html) |
| *P. tomopterna* | TO1 | PLS-TO1 (PS-8) | **FLSLIP**HAINAVSALAKHF-$NH_2$ | P85447 (http://www.uniprot.org/uniprot/P85447.html) |

The bold residues are conserved

[a] The peptides are named according to the nomenclature proposed by Amiche et al. (2008), the original nomenclature are in brackets

The antimicrobial activity spectrum of PTC-B1 includes strains of Gram-negative bacteria: *Clostridium perfringens*, *Escherichia coli*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Listeria monocytogenes*, *Neisseria meningitidis*, *Pseudomonas aeruginosa*, *Salmonella enteritidis*, and *Vibrio cholerae*; Gram-positive bacteria: *Bacillus megaterium*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Staphylococcus haemolyticus*, and *Streptococcus pneumoniae*; and Fungi: *Candida albicans* and *Saccaromyces cerevisiae* (Vanhoye et al. 2004). Haemolysis was not detected (Vanhoye et al. 2004). The Gram-positive bacteria *Burkholderia cepacia* is resistant to PTC-B1 (Vanhoye et al. 2004). Without C-terminus amidation of PTC-B1, antimicrobial activity ceases, except for *Staphylococcus aureus* and *S. haemolyticus* that are more affected by PTC-B1 40-48 folds than the PTC-B1 amide. Hemolytic activity was recorded (Vanhoye et al. 2004), supporting data from Matsuzaki (2009) in that the reduction of the peptide positive net charge reduces its antimicrobial activity.

### Skin polypeptide YY

Related peptides that belong to the Neuro Peptide Y (NPY) family (36 residues length) which also include peptide YY (PYY), the tetrapod pancreatic polypeptide (PP), and the fish pancreatic peptide Y (PY) have been found in various vertebrate groups (Lazarus and Attila 1993; Cerdá-Reverter and Larhammar 2000). These peptides integrate a variety of important regulatory functions, e.g., sympathetic vascular control, central regulation of endocrine and autonomic function, food intake, circadian rhythm, histamine release from isolated mast cells, and increase of intracellular $Ca^{2+}$ in many cell types (Yasuhara et al. 1981).

In *Phyllomedusa*, the only peptide pharmacologically and structurally related to NPY described was the skin polypeptide YY (SPYY) (Mor et al. 1994a). SPYY was purified from acetic extracts of *Phyllomedusa bicolor* skin (Mor et al. 1994b), exhibiting 94% of similarity with PYY from the frog *Rana ridibunda* (Conlon et al. 1992) and 86% of similarity with human PYY (Kohri et al. 1993) (Table 10). The primary structures of the two frog NPYs are highly conserved presenting only two amino acid substitutions (positions 7 and 18) (Table 10).

Besides the NPY–RP primary structures similarity, other common features are the C-terminal amidation and the tertiary structure, known as the PP-fold (Erspamer et al. 1962). The PP-fold consists of two antiparallel helices: an N-terminal polyproline helix spanning residues 1–14 and a long amphipathic C-terminal α-helix.

As other peptide hormones of the amphipathic helix class with PM disordering or disruptive properties, such as glucagon (Jones et al. 1978), SPYY also shows PM lysing activity against pathogenic microbes. SPYY shows antibiotic activity against strains of Gram-negative bacteria: *Aerornonas caviae* and *Escherichia coli*; Gram-positive bacteria: *Enterococcus faecalis* and *Nocardia brasiliensis*; Fungi: *Arthroderma simii*, *Aspergillus fumigatus*, *A. niger*, *Microsporum canis*, and *Tricophyton rubrum*; and Protozoa: *Leishmania major* promastigotes. Reversibility of inhibition was not reported for any strain (Vouldoukis et al. 1996).

## Therapeutic peptide antibiotics

One of the greatest accomplishments of modern medicine was the development of antibiotic therapies for potentially fatal infections by multidrug-resistant pathogenic microorganisms. Unfortunately, in the past two decades, the discovery and development of novel antibiotics decreased while pathogen resistance to those currently available increased (Li et al. 2006).

**Table 8** Primary structures of phylloxins from *Phyllomedusa* species

| Species | Phylloxin | Abbreviation | Sequence | Digital abstract |
|---|---|---|---|---|
| *P. bicolor* | B1 | PLX-B1 | **GWMSKIASGIGTFLSG**M**QQ**-$NH_2$ | P81565 (http://www.uniprot.org/uniprot/P81565.html) |
| *P. sauvagii* | S1 | PLX-S1 | **GWMSKIASGIGTFLSG**V**QQ** | Q5DVA6 (http://www.uniprot.org/uniprot/Q5DVA6.html) |

The bold residues are conserved

**Table 9** Primary structures of plasticins from *Phyllomedusa* species

| Species | Plasticin[a] | Abbreviation[a] | Sequence | Digital abstract |
|---|---|---|---|---|
| *P. bicolor* | B1 | PTC-B1 (PBN2) | **G**L**V**TS**L**IKGAGK**LLG**G**L**F**G**SVTG-$NH_2$ | Q800R4 (http://www.uniprot.org/uniprot/Q800R4.html) |
| *P. sauvagii* | S1 | PTC-S1 (DS 10) | **G**L**V**SD**L**LSTVTG**LLG**N**L**G**G**GGLKKI | Q1EN14 (http://www.uniprot.org/uniprot/Q1EN14.html) |

The bold residues are conserved

[a] The peptides are named according to the nomenclature proposed by Amiche et al. (2008), the original nomenclature are in brackets

The emergence and rapid spread of extremely multiresistant pathogenic microorganisms, the increased use of immunosuppressive therapies, and the association with HIV co-infection present a serious public health problem with worrisome mortality and morbidity rates (e.g., *Cryptococcus*, *Cryptosporidium*, and *Leishmania*) (Abu-Raddad et al. 2006; Pukkila-Worley and Mylonakis 2008; Rivas et al. 2009; Vaara 2009). Limited therapeutic options against these pathogens stimulated the prospection of new bioactive molecules from the biodiversity as a source for more efficient (low toxicity and major potency) mechanisms of microorganism killing (Calderon et al. 2009a; Vaara 2009). This information is important to subsidize the development of new chemicals with structural characteristics for large-scale production by the pharmaceutical industry at a feasible cost. The sources from the biodiversity, such as the skin of several frogs' species, e.g., as *Phyllomedusa* and other vertebrate and invertebrate animals, plants, and microorganisms, have proved to be an inexorable source of antimicrobial molecules, with a broad spectra of activity (Calderon et al. 2009a), in which the AMPs have highlights in their potential therapeutical application (Hancock 1997; Hancock and Lehrer 1998; Koczulla and Bals 2003; Gomes et al. 2007). During the last 40 years of antimicrobial peptides research, lots of information were generated, with insights about key issues of the peptide antimicrobial potency and selectivity, allowing the development of synthetic rational designed peptides with improved antimicrobial activity (Andrä et al. 2007) and less toxicity to mammalian cells (Hawrami et al. 2008) by the application of site-directed mutation, combinatorial chemistry, and chemical synthesis techniques (Hilpert et al. 2006; Edwards 2007; Andrä et al. 2007). In order to develop new peptide antibiotics, synthetic changed peptides were designed including: improvement of positive charge, decreasing-induced resistance in bacteria (Zasloff 2002; Andreu and Rivas 1998; Hancock and Lehrer 1998; van't et al. 2001; Moellering 2003; Yeaman and Yount 2003); lower molecular mass by reduced number of amino acid residues (Hancock 1997; Boman 2003; Perron et al. 2006; Peschel and Sahl 2006; Bisht et al. 2007; Haug et al. 2007); and insertion of unnatural amino acids (Edwards 2007). All peptide modifications might offer significant advantages over native AMPs as therapeutical agents (Rotem and Mor 2009).

According to Marr et al. (2006), therapeutic peptide antibiotics will have advantages over conventional antibiotics due their diverse potential applications, such as single antimicrobials, in combination with other antibiotics for a synergistic effect, or as immunomodulatory and/or endotoxin-neutralizing compounds (Zasloff 2002). In particular, the most potent agents have unusually broad spectra of activity against most Gram-negative and Gram-positive bacteria, and also to fungi and even a variety of viruses. Although the potency of these AMPs against the more susceptible pathogens is generally less than certain conventional antibiotics, one of their advantages is their ability to kill multidrug-resistant bacteria at similar concentrations (Marr et al. 2006). Compared with conventional antibiotics, these bacteria-killing peptides are extremely rapid and attack multiple bacterial cellular targets (Brogden 2005).

Despite their obligatory interaction with the PM, some peptides are able to perforate PMs at their minimal inhibition concentration (MIC), a number of peptides translocate across the PM and affect cytoplasmic processes, including inhibition of macromolecular synthesis, particular enzymes or cell division, or the stimulation of autolysis (Marr et al. 2006). Minimal inhibitory concentrations and minimal bactericidal concentrations often coincide (less than a two-fold difference), indicating that killing is generally bactericidal, a highly desirable mode of action (Marr et al. 2006). Furthermore, peptides are not hindered by the resistance mechanisms that occur with currently used antibiotics (Zhang et al. 2005). Indeed, killing can occur synergistically with other peptides and conventional antibiotics, helping overcome some barriers that resistant bacteria have against currently used antibiotics (Marr et al. 2006).

## Nanobiotechnological application of *Phyllomedusa* AMPs

In recent years, significant efforts were devoted to the development of nanotechnological tools capable of

**Table 10** Primary structures of polypeptide YY from *Phyllomedusa bicolor*, *Rana ridibunda*, and human

| Species | Name | Sequence | Digital abstract |
|---|---|---|---|
| *P. bicolor* | Skin polypeptide YY (SPYY) | **YP**P**KPE**S**PGEDASPEEMNKY**LTA**LRHYINLVTRQRY**-$NH_2^a$ | P80952 (http://www.uniprot.org/uniprot/P80952.html) |
| *R. ridibunda* | Peptide YY-like (PYY) | **YP**P**KPE**N**PGEDASPEEM**T**KY**LTA**LRHYINLVTRQRY**-$NH_2$ | P29204 (http://www.uniprot.org/uniprot/P29204.html) |
| *H. sapiens* | Peptide YY (PYY) | **YP**I**KPE**A**PGEDASPEELNRY**YAS**LRHYLNLVTRQRY**-$NH_2$ | P10082 (http://www.uniprot.org/uniprot/P10082.html) |

The bold residues are conserved

[a] The C-terminal amidation given is based on similarity and not on experimental findings

enhancing the assembly and immobilization of biomolecules in a synergistic way in biomedical devices (Huguenin et al. 2005; Siqueira et al. 2006; Zucolotto et al. 2006; Zampa et al. 2007; Zucolotto et al. 2007). Nanotechnology focuses on formulating therapeutic agents in biocompatible nanocomposites, such as nanoparticles, nanocapsules, micellar systems, and conjugates. As these systems are often polymeric and submicron sized, they have multifaceted advantages in drug delivery.

The structural and physico-chemical properties of the AMPs, such as the presence of a α-helix structure and distribution of positive charges along the chain, allowed their use as active material in the development of bionanostructures with potential application on therapeutics by the pharmaceutical industry and diagnosis (Zampa et al. 2009). These structures include cationic nanoparticles, formed by the conjugation of cholesterol and AMPs, able to cross the blood–brain barrier for treatment of fatal *Cryptococcal* meningitis in patients with late-stage HIV infection (Wang et al. 2010); nanostructured thin films with immobilized AMPs as an agent intended to combat and prevent infection and formation of *Staphylococcus* biofilm (slimelike communities) related implant failure (Shukla et al. 2009); or as sensor elements for detection of *Leishmania* cells using cyclic voltammetry (Zampa et al. 2009).

The use of the AMPs through nanotechnological innovation approach could provide an entirely novel way to treat and prevent infection and new systems for the detection and identification of infectious parasites.

## Final considerations

The *Phyllomedusa* skin is an abundant source of peptides that show a broad spectrum of activities, including antimicrobial, neuroactive, and smooth muscle activity. From the first *Phyllomedusa* peptide isolated and characterized to date, more than 200 peptides from *Phyllomedusa* species have had their primary structure characterized, and several of them had its biological activities evaluated, mainly in the last 10 years. Until then, many efforts have been carried out in order to use the AMPs in the development of new infection-fighting drugs applicable to new treatments of nosocomial infections and multidrug-resistant infections (Amiche et al. 2000), due to the skill of the AMPs to kill drug resistant strains of Gram-positive bacteria, Gram-negative bacteria, yeast, protozoa, and viruses, by a mechanism unlikely to induce antibiotic-resistance. The development of new antimicrobials based on AMPs hold promises to medicine at the end of the classical antibiotic age by the emergence of the multidrug-resistant microorganisms.

Even with the expected advantages in the use of AMPs as antibiotics, several impediments to therapeutic peptides arise. According to Marr et al. (2006), the main problems at the present moment are the cost of manufacturing peptides, which is economically unfeasible for the amounts of AMPs needed compared to other antibiotics, preventing the widespread clinical use of AMPs as a common antibiotic, and the shortage of studies thoroughly examining systemic peptide pharmacodynamic and pharmacokinetic issues, including peptide aggregation problems, the in vivo half-life of peptides (and particularly their susceptibility to mammalian proteases), and the required dosing frequency.

Due to the specific characteristics of the AMPs, that differentiate them from other antibiotics, the development of new strategies for the therapeutic use of AMPs in medicine are necessary in order to reduce the amount of AMPs necessary to promote the therapeutic infection suppression effect, including the addition of striking affinity to specific targets, efficiency at very low concentrations and negligible toxicity. In this way, nanotechnology has become an efficient and viable alternative to promote the therapeutic application of AMPs. Nanotechnology could provide new ways to use lower amounts of AMPs with extreme efficiency in the infection suppression, by improving the cell, tissue, or organ's specific biodistribution and increasing AMP potency by the association with nanotechnological structures. It is expected that in the forthcoming years nanotechnology will promote the emergence of new products for control and prevention of multidrug-resistance microbe infection arising from the identification and analysis of AMPs from South American frog biodiversity.

**Acknowledgments** The authors are grateful to the Ministry of Science and Technology (MCT), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Financiadora de Estudos e Projetos (FINEP), Fundação de Tecnologia do Acre (FUNTAC/FDCT), Coordenação de Aperfeiçoamento de Nível Superior (CAPES) – Projeto NanoBiotec, Secretary of Development of the Rondonia State (PRONEX/CNPq) for financial support and Priscila Cerviglieri for linguistic advice.

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