Jordan Journal of Pharmaceutical Sciences, Volume 9, No. 1, 2016

A Review of Antifungal Peptides: Basis to New Era of Antifungal Drugs

Yazan Akkam [1]*

[1] Faculty of Pharmacy, Yarmouk University, Irbid, Jordan.

ABSTRACT

The limited arsenal of antifungal drugs coupled with the growing number of drug resistant isolates of Yeast pathogens stresses the need for the development of new antifungal agents with new mechanisms of action. Antimicrobial peptides, an active arm of the innate immunity, represent the natural arsenal against pathogens including the yeast. This review is focused on studying the biophysical prosperities (net charge, stereospecificty, conformation, amphipathicity, hydrophobicity, and length) of the peptides that exhibit antifungal activity enclosed within the antimicrobial peptides database till September/2015. Studying these peptides may be the key for discovering and developing of new potent antifungal agents as they have served as models in drug discovery. Furthermore, the review discusses the main obstacles and barriers -specifically the salt sensitivity- that prevents antifungal peptides from becoming successful drug stories. Proposed solutions from industry and nature are also discussed. This review serves as a potential base for utilizing antifungal peptides in drug discovery.

Keywords: Antimicrobial peptides, Antifungal peptides, Hydrophobicity, Amphibathicity, Cationic peptides, Anionic peptides, Salt intolerance.

1. INTRODUCTION

The extensive use of current available antifungal drugs has resulted in the appearance of drug resistant strains and their incidence is increasing drastically. For instance, resistant yeast to azoles[1-5], polyenes[6-10], and echinocandins[11-15] have been isolated. Also uncommon human fungal pathogens, which by nature resist current antifungal drugs, start gaining further attention especially in immunocompromised patients such as *Aspergillus* spp (flavus and fumigatus)[16], *zygomycetes* spp (mucorales and entomophthorales)[17], *Fusarium* spp (solani and oxysporum)[18], and *Scedosporium* spp (prolificans and aurantiacum)[19, 20]. Given the morbidity and mortality associated with yeast infections, there is a strong emphasis on the development of new anti-fungal drugs with novel mechanisms-of-action [21].Concequently, the newly designed antifungal drugs would be less likely to be affected by current resistance mechanisms or subjected to the possibility of cross-resistance. The average length of time for a drug to reach the market and get the FDA approval is approximately 15 years[22]. Therefore, the current focus on developing new antifungal drugs is a logic step before our current arsenal of drugs fail.

This review discusses peptides featuring antifungal activities (antifungal peptides-AFPs) as antifungal agents with focusing on studying the biophysical characteristics that may interfere with the antifungal activity; net charge, stereospecificity, hydrophobicity, amphipathicity, secondary structure, and peptide length. Furthermore, this review presents the post-translation modification in the natural antifungal peptides as a lesson from nature to improve the activity. The obstacles preventing AFPs from reaching the market were also addressed with emphasis on the salt sensitivity. Finally, salt resistance peptides were summarized and analyzed.

* yazan.a@yu.edu.jo

Received on 28/9/2015 and Accepted for Publication on 7/1/2016.

2. Antimicrobial and Antifungal Peptides

Antimicrobial peptides (AMPs) are known to play an important role in the human innate immune response against pathogenic and opportunistic microorganisms[2]. The AMPs also serve as promising candidates for new therapeutic compound as they possess unique features; broad activity, rapid action, low microbial resistance and high selectivity. Consequently, several synthetic and semi-synthetic peptide -based- drug have been synthesized[23].

The broad activity of AMPs makes it difficult to exclusively classify them as antifungal or antibacterial. There are relatively few examples where a peptide retains solely antifungal or antibacterial activities. In some cases, AMPs could be antibacterial; however, their antifungal activities have not been tested yet, and vice versa. This review will discuss antimicrobial peptides that exhibit anti-fungal activities and they will be referred to as antifungal peptides[24-28].

The AMPs which include AFPs represent a diverse array of sequences, and there could be no perfect way to classify them. Several reviews have classified the AMPs using different criteria: secondary structures[29, 30], source and the mechanism of action[26, 30, 31], cells that produce the peptide[32, 33,34], post-translation modification [35] and the species (eukaryotic and prokaryotic)[36, 37].

3. The antimicrobial peptide database:

To get a broad picture of currently available AFPs, the antimicrobial peptide database (http://aps.unmc.edu /AP/main.php)[38] was mined till September/2015 for all possible candidates. In the database search engine, only Fungi were selected under Antimicrobial Activity section and everything else was left empty. Subsequently, around 940 AMPs exhibiting antifungal activities were resulted and then studied. To calculate the percentage of each biophysical characteristic within the database, the search engine tools were utilized.

4. Biophysical properties of AFPs

This section discusses the main biophysical characteristics of AFPs in general which includes net charge, stereospecificity, hydrophobicity, amphipathicity, secondary structure, and peptide length. It is important to mention that some of these characteristics are interdependent; therefore, modification of one character may lead to alterations in the other.

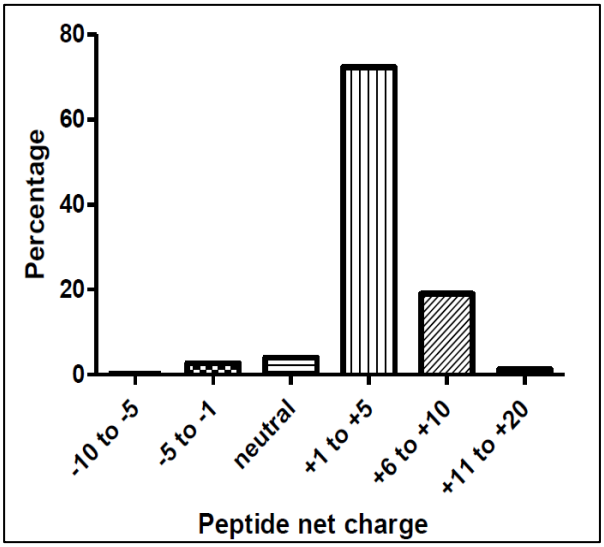

Figure 1: Distribution of the net charge within anti-fungal peptides. The data were generated using the antimicrobial peptide database

4.1 Charge

According to the net charge, the AFPs can be classified into three categories; cationic, neutral, and anionic peptides. The vast majority of database available AFPs are cationic (Figure 1) and display a positive net charge which ranges between +1 to +15 (Table 1).

In cationic AFP, the positive charge plays an important role whereby an electrostatic binding to the negatively charged membrane is initiated. However, increasing the positive charge does not always improve the antifungal activity, it depends on the peptide sequence and secondary structure[39]. Hence, the increase in positive net charge has shown different effects on the antifungal activity. For example, increasing the number of lysine residues within dF17-6K (compare with dF21-10K) improved the antifungal activity[40]. On the other hand, increasing the charge within MtDef4 peptide from +6 to +7 had an inhibitory effect on the activity against *F.*

graminearum[41]. It has been demonstrated that increasing peptide positive net charge beyond a threshold might lead to a strong interaction with the negatively charged membrane, resulting in an inhibition in peptide translocation into the cell[42]. In some peptides, the amount of the positive charge and the net charge was not as important as the location of the cationic residue[39].

Table 1. Examples of cationic AFPs sorted in ascending order by the net charge

Name	Origin	Amino acid sequence	Activity	Secondary structure PDB	Net Charge	Ref.
Heliomicin	*Heliothis virescens (worm)*	DKLIGSCVWGAVNYTSDCNGECKRRGY KGGHCGSFANVNCWCET	*C. albicans* *C. neoformans*	Helix and Be1I2U	+1	144
Metchnikowin	*Drosophila melanogaster*	HRHQGPIFDTRPSPFNPNQPRPGPIY	*F. graminearum*	unknown	+2	145
Maximin 1	*Bombina maxima*	GIGTKILGGVKTALKGALKELASTYAN	*C. albicans*	unknown	+3	146
Ranatuerin 1	*Rana catesbeiana*	SMLSVLKNLGKVGLGFVACKINKQC	*C. albicans*	unknown	+4	147
Melittin	*Apis mellifera*	GIGAVLKVLTTGLPALISWIKRKRQQ	*C. albicans*	Helix 1MLT	+5	148
Tachystatin A2	*achypleus tridentatus*	YSRCQLQGFNCVVRSYGLPTIPCCRGLT CRSYFPGSTYGRCQRY	*C. albicans*	Beta 1CIX	+6	149
MBP-1	*Zea mays L*	RSGRGECRRQCLRRHEGQPWETQECMR RCRRRG	*F. graminearum* *F. moniliforme*	Helix[1]	+7	150
Lactoferricin B	*Bos Taurus*	FKCRRWQWRMKKLGAPSITCVRRAF	*C. albicans* *T. mentagrophytes* *T. rubrum*	Beta 1LFC	+8	151
1AFP	*Aspergillus giganteus*	ATYNGKCYKKDNICKYKAQSGKTAICK CYVKKCPRDGAKCEFDSYKGKCYC	*F. sambucinum* *N. crassa* *A. niger*	Beta 1AFP	+9	152
BMAP-27	*Bos taurus*	GRFKRFRKKFKKLFKKLSPVIPLLHLG	*C. albicans* *C. neoformans*	Helix 2KET	+10	153
sBD-1	*Ovis arues*	NRLSCHRNKGVCVPSRCPRHMRQIGTC RGPPVKCCRKK	*C. albicans*	unknown	+11	154
Buforin I	*bufo gargarizans*	AGRGKQGGKVRAKAKTRSSRAGLQFPV GRVHRLLRKGNY	*C. albicans* *S. cerevisiae* *C. neoformance*	unknown	+12	155
CXCL14	*Homo sapiens*	SKCKCSRKGPKIRYSDVKKLEMKPKYP HCEEKMVIITTKSVSRYRGQEHCLHPKL QSTKRFIKWYNAWNEKRRVYEE	*C. albicans*	unknown	+13	156

CodCath	*Gadus morhua*	SRSGRGSGKGGRGGSRGSSGSRGSKGPS GSRGSSGSRGSKGSRGGRSGRGSTIAGN GNRNNGGTRTA	*C. albicans*	unknown	+15	157

Table 2. Anionic AFPs sorted in ascending order by the net charge

Name	Origin	Amino acid sequence	Activity	Secondary structure PDB	Net Charge	Ref.
Tn-AFP	*Trapa atans*	LMCTHPLDCSN	*C.tropicalis*	Unknown	-1	158
An-AFP	*Aspergillus niger*	SKYGGECSVEHNTCTYLKGGKD HIVSCPSAANLRCKTERHHCEYD EHHKTVDCQTPV	*C. albicans, S. cerevisiae T. beigelii, F. solani F. oxysporum, A. fumigatus A. flavus*	Unknown	-1	159
Kalata B1	*Viola betonicifolia*	GLPVCGETCFGGTCNTPGCTCT WPICTRD	*C. kefyr*	Helix and Beta 1PT4	-1	160, 161
PvD1	*Phaseolus vulgaris*	KTCENLADTYKGPCFTTGSCD	*C. albicans, C. parapsilosis, C. tropicalis, C. cerevisiae*	Expected Helix and Beta	-1	162, 163
human Dermcidin	*Homo sapiens*	SSLLEKGLDGAKKAVGGLGKLG KDAVEDLESVGKGAVHDVKDV LDSV	*C. albicans*	Helix-2KSG	-2	52
EP-20	*Xenorhabdus budapestensis*	EGPVGLADPDGPASAPLGAP	*P. capsici V. dahliae*	Unknown	-3	44
Beta-amyloid peptide	*Homo sapiens*	DAEFRHDSGYEVHHQKLVFFAE DVGSNKGAIIGLMVGGVV	*C. albicans*	Helix 1IYT	-3	51
Ls-Stylicin1	*Litopenaeus stylirostris*	SSFSPPRGPPGWGPPCVQQPCPK CPYDDYKCPTCDKFPECEECPHI SIGCECGYFSCECPKPVCEPCESP IAELIKKGGYKG	*F. oxysporum*	Unknown	-3	164
Gm anionic peptide-2	*Galleria mellonella*	EADEPLWLYKGDNIERAPTTAD HPILPSIIDDVKLDPNRRYA	*P. pastoris P. stipites C. albicans C. fructus Z. marxianus*	Unknown	-4	165

Microplusin	*Rhipicephalus (Boophilus) Microplus*	HHQELCTKGDDALVTELECIRLR ISPETNAAFDNAVQQLNCLNRA CAYRKMCATNNLEQAMSVYFT NEQIKEIHDAATACDPEAHHEH DH	*S. cerevisiae C. neoformans*	Helix 2KNJ	-8	166

Although the vast majority of AFPs families are cationic, a few are neutral (table 2). For example, Aurein 1.1 is neutral; whereas, the other 11 Aurin peptides are cationic. The brevinin family is another example where brevinin-1- OR3, OR6, OR8, and OR9 are neutral peptides and the rest are cationic[43]. An exceptional case is GP-19 peptide, which is neutral, and GP-20 peptide is anionic[44]. Despite the net charge is neutral, It has been suggested that the positive charges within the neutral peptides are still able to initiate enough electrostatic interactions with the negatively charged membrane[45 44 46]. This theory does not fit with Gp-19 and Temporin-1PRb peptides as their sequence lack charged amino acids, and they exhibit antifungal activities.

The first anionic AMPs were described in 1984[47] and after that several anionic peptides were identified in both eukaryotes and prokaryotes[48, 49]. Just like cationic AMPs, anionic AMPs might play an important role in the innate immunity[50]. Out of 116 anionic peptides described in the peptide database, only 11 peptides have shown antifungal activity with net charges range from -1 to -8 at neutral pH (table 3). Unlike cationic peptides, anionic peptides display a negative net charge that could lead to repulsion with the negatively charged membrane. It has been found-in anionic peptides- that the overall positive charge is not a prerequisite for the binding to the membrane, and the key modulators of lipid bilayers-peptide interaction is the charge distribution and the secondary structure[51]. Consequently, the anionic peptides might be able to interact with the membrane because their basic amino acids are distributed in such a way to give them the accessibility and limit the repulsive effect of negatively charged residues. This model does not fit all anionic peptides since some of these peptides do not have a single basic amino acid, such as Tn-AFP and EP-20. Surprisingly, these peptides retain the capability of binding to the target membrane, and this binding is critical for the activity[50,52]. The other suggested explanation is that the anionic peptides form cationic salt bridges with membrane negative charges via metal ions[49,50, 52].

4.2. Stereospecificity

Stereospecificity means certain biological processes or chemical reactions are specified for only one of several possible stereoisomers. Within peptides, the focus is on enantiomers or optical isomers. Generally, stereospecificity in the binding processes is an essential requirement for peptide or protein-target interaction and lacking this characteristic might suggest a lack of overall specificity[53].

In AMPs, binding to the target microorganism is required to achieve an activity; however, the majority of the AMPs are not stereospecific with some exceptions[54]. Several publications have shown that all-D-amino acid peptides demonstrated similar antimicrobial activities as their all-L-enantiomers[55-58]. As a subgroup of AMPs, AFPs exhibited the same characteristic[55, 59] and multiple examples were published demonstrating the stereoisomeric AFPs were equal in activity[55, 59]. For example, all-D variants of the histatin peptide fragments (including P-113) are equally internalized and they exhibited the same activity. Therefore, the involvement of a stereospecific receptor was excluded[60, 61]. However, in some case the stereospecificity may interfere with the AFPs activity but at a lesser extent than AMPs. The thanatin peptide is an example of a stereospecific AMP, where the activity against gram-positive bacteria was inhibited in all-D enantiomers, but the activity was retained against fungi just like L-thanatin[62]. The Bac7 peptide, a cathelicidin derived peptide, is a unique example where its antifungal activity shows some

sterospecificity. It has been shown that Bac7 exhibits a stereospecific binding to *C. neoformans* membrane at concentrations near the MIC values, but this interaction became non-stereoselective at higher concentrations[63]. In conclusion, despite the net charge, the vast majority of AFPs are not stereo-specific.

4.3. Conformation

Similar to other AMPs, there is no dominant conformation within the AFPs; they differ in sequence and secondary structure. The antifungal peptides could be defined according to their conformation into five categories: α- helix, β-hairpen or sheet, mixed α- helix/β-sheet, amino acid rich peptides, and unknown (not determined yet) (Figure 2).

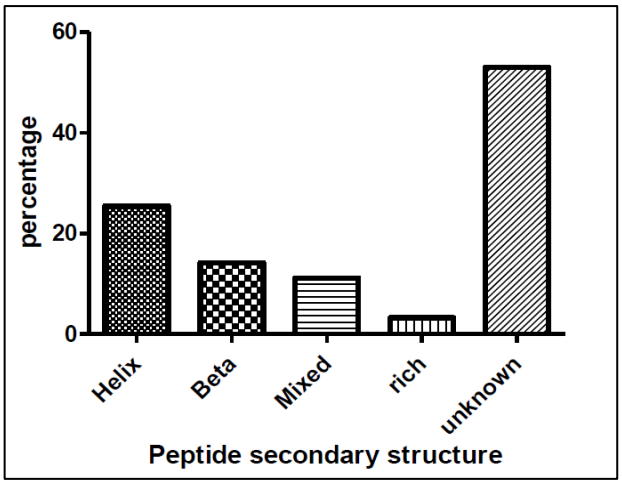

Figure 2: Distribution of the secondary structure within anti-fungal peptides.

Unfortunately, the conformations of more than 50% of AFPs found in the database have not been determined yet. Within the remaining 50%, α-helices are the abundant within AFPs; however, they frequently exist as unstructured conformers. These peptides become helical only upon interaction with an amphipathic membrane such as a fungal plasma membrane[64]. The β-sheet containing AFPs are less abundant than α- helix, and this category is highly diverse at the level of primary structure but they share common features, such as the amphipathic structure[42]. While studying the peptides within the antimicrobial database, it has been noted that AFPs with beta structure share a common feature; containing at least 2 cysteine residues. This feature is not always present in AMPs since there are beta structured AMPs without a disulfide bridge[65-68].

Most amino acid-rich AFPs could be further divided into one of the following subgroups: Gly-rich, Pro-rich, Arg-rich, His-rich, and Trp-rich. According to the antimicrobial peptide database, AFPs did not contain any Lys-rich peptides which typically found in AMPs, such as the dermaseptin family [69] and GLK-19 [38]. AFPs enriched in particular amino acids exhibited different conformations forming unusual helices or sheets. For instance, tritrpticin, a tryptophan –arginine rich peptide, has retained multiple structures upon binding to a micelle, including a turn-turn and extended α-helix structure[70]. SP-B, a proline-rich peptide exhibited both antibacterial and antifungal activity, is composed of polyproline-II helices, unordered and turn motifs[71].

The secondary structure is very diverse with abundance of alpha helix in all classified peptides (anionic, neutral and cationic AFPs) (Table 3).

4.4. Amphipathicity

Amphipathicity is defined as the ability of a molecule to adopt a shape or structure in which clusters of hydrophobic and hydrophilic amino acids are spatially organized in discrete sectors[29]. Most of the AMPs as well as the AFPs form amphipathic structures upon interaction with target membranes. Amphipathicity for a peptide is usually determined by the hydrophobic moment (μH)[42], which is the hydrophobicity of a peptide measured for different angles of rotation (0-180 degree) per residue[72]. Measuring the μH for a peptide assists in recognizing amphipathic structures via determining when the residues on one side of the structure are more hydrophobic than on the other[73].

Regardless the net charge of the AFPs and secondary structure, with some exceptions, increasing the hydrophobic moment leads to increase the antifungal activity via promoting membrane permeabilization. For

instance, In P19(6/E) peptide, research has shown the reduction of amphipathicity by scrambling the sequence was enough to reduce the antifungal activity against *C. albicans* and *C. neoformans,* even though the amino acid composition, charge, mean hydrophobicity and helical propensity were the same[74].

Table 3. Examples of neutral AFPs. These examples represent the vast majority of neutral AFPs within the database

Name	Origin	Amino acid sequence	Activity	Secondary structure PDB	Ref.
Aurein 1.1	*Litoria raniformis*	GLFDIIKKIAESI	*C. albicans,* *C. Tropicalis* *C. Krusi,* *C. Parapsilosis* *C. glabrata*	Helix 2F3A	167
Maculatin 1.1	*Litoria genimaculate*	GLFVGVLAKVAAHVVPAIAEHF	*C. albicans*	Helix	168
Skin tyrosine peptide	*Phyllomedusa bicolor*	YPPKPESPGEDASPEEMNKYLTALRHYINLVTRQRY	*C. albicans,* *A. fumigatus* *C. neoformans*	unknown	169
-Galleria defensin -Galleria defensin like	*Galleria mellonella*	DTLIGSCVWGATNYTSDCNAECKRRGYKGGHCGSFLNVNCWCE DKLIGSCVWGATNYTSDCNAECKRRGYKGGHCGSFWNVNCWCEE	*C. albicans,* *G. candidum* *C. neoformans,* *F. oxysporum*	unknown	170 165
Gm cecropin D-like peptide	*Galleria mellonella*	ENFFKEIERAGQRIRDAIISAAPAVETLAQAQKIIKGGD	*A. niger*	unknown	165
Histatin 2	*Homo sapiens*	RKFHEKHHSHREFPFYGDYGSNYLYDN	*C. albicans*	unknown	171
Temporin-1PRb	*Rana pirica*	ILPILGNLLNSLL	*C. albicans*	unknown	172
Neuropeptide Y	*Homo sapiens*	YPSKPDNPGEDAPAEDMARYYSALRHYINLITRQRY	*C. neoformans,* *C. albicans* *C. krusei, C. utilis*	Helix 1RON	45 173
GHH20	*Homo sapiens*	GHHPHGHHPHGHHPHGHHHPH	*C. parapsilosis,* *C. albicans*	Helix	98
Ha-DEF1	*Helianthus annuus*	ELCEKASQTWSGTCGKTKHCDDQCKSWEGAAHGACHVRDGKHMCFCYFNC	*S. cerevisiae*	unknown	174
Drosomycin-2	*Drosophila melanogaster*	DCLSGKYKGPCAVWDNEMCRRICKEEGHISGHCSPSLKCWCEGC	*N. crassa,* *G. candidum* *S. cerevisiae*	Helix Beta-1MYN	175

Sm-AMP-D1	*Stellaria media L*	KICERASGTWKGICIHSNDCNN QCVKWENAGSGSCHYQFPNY MCFCYFDC	*Phytopathogenic fungi*	unknown	176
Brevinin-1-OR3	*Odorrana rotodora*	IDPFVAGVAAEMMQHVYCAA SKKC	*C. albicans*	unknown	43
Andersonin-X1	*Odorrana andersonii*	GLFSKFAGKGIVNFLIEGVE	*C. albicans*	unknown	43
GP-19	*Xenorhabdus budapestensis NMC-*10	GPVGLLSSPGSLPPVGGAP	*F. omysporum, P. capsici V. dahlia, F.graminearum*	unknown	44

Most of the α-helical AFPs have amphipathic structures, but some are not. For example, kaxins is a class of AFPs that do not exhibit an amphipathic structure[40, 75]. This class has displayed antifungal activity with minimum hemolytic activity, and that supports the correlation between peptide amphipathicity and hemolysis[40].

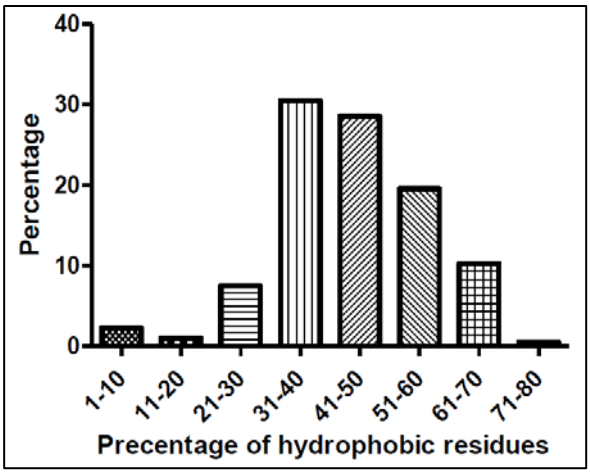

Figure 3: Percentage of the hydrophobic residues within anti-fungal peptides.

The amphipathicity is also observed in β-structures. Tachyplesin and polyphemusin are examples of amphipathic β-sheet[76] while Rhesus theta defensin-1 (RTD-1) peptide is an example of non-amphipathic β-sheet[77]. In amino acid -rich peptides, the amphipathic structure was also perceived such as in histatin 5(His-rich)[78] and Pac-525 (Trp-rich)[79]. It is also important to mention that all proline-arginine rich peptides (AMP or AFP) cannot form amphipathic structures due to the formation of unusual secondary structure, polyproline helical type-II[80].

Increasing the amphipathicity does not only interfere with the antifungal activity, it surges the hemolytic activity. For the development of new peptides, especially with membrane lytic mechanisms, it has been suggested to keep μH less than 0.3[81].

The bottom line, amphipathicity in AFPs seems important in the vast majority, but not a must, it totally depends on the peptide sequence. For instance, the reduction of YLK peptide amphipathicity by utilizing helix breaker residues enhanced the antifungal activity[81].

4.5. Hydrophobicity

Peptide hydrophobicity is defined as the percentage of hydrophobic residues within a peptide length. The vast majority of antifungal peptides have hydrophobic values ranging between 30-60% (Figure 3). Hydrophobicity is an essential requirement for peptide membrane interactions as well as membrane permeabalization. Furthermore, the peptide partitioning into the phospholipids layer is also controlled by peptide hydrophobicity. Increasing peptide hydrophobicity might correlate with an increase in the activity, but it also has been linked to increased hemolysis activity[42, 82]. For example, increasing the hydrophobicity of the D1 peptide, to generate a peptide termed D4, led to the

induction of hemolytic activity by approximately 286-fold. In the same example, the modification of the D1 peptide also interfered with the fungicidal activity. The D1 peptide was more active against *Zygomycota* fungi while the D4 peptide was more active against *Ascomycota* fungi[42, 82].

Lipopeptides is a class of antifungal peptide where lipophilic moieties are attached to cationic peptides. Although this class is very hydrophobic, they had a low hemolysis activity[83].

It should also be noted that hydrophobicity is not the only factor related to hemolysis, the presence of tryptophan was also linked to the hemolytic activity. A study has shown that replacing one asparagine residue with tryptophan in the NDGP peptide was enough to increase the hemolytic activity approximately 24 fold[81]. Further, it has been concluded that tryptophan - tryptophan interactions and tryptophan - lipid interactions are responsible for the increase in hemolytic activity of melittin-tryptophan analogs rather than the hydrophobicity[81]. It has been published that tryptophan has a strong ability to insert into membranes as well as to interfere with lipid polymorphism[84]. In spite of the role of tryptophan in hemolytic activity, it has been inspected in multiple research studies and the results were varied. For instance, the Pac-525 peptide is a tryptophan-rich peptide that exhibits low hemolysis activity[79].

In general, hydrophobicity is essential to AFPs especially membrane acting peptides, and does not change much by net charge and secondary structure; however, there are some exceptions.

4.6. Peptides Length

Total number of amino acids plays an important role in the secondary structure and mode of action of AFPs. Peptides less than 20 amino acid are less possible to cross the yeast membrane in an α-helix structure and thereby designate the mode of action[85]. As illustrated in figure-4, the majority of AFPs are between 11to 40 amino acids. But in some AFPs the full length is not essential to achieve full activity as smaller fragments of the peptide exhibit the full activity[86-88]. Therefore, the trend now is to design a short peptide to increase the cost effectiveness. The AFPs that passed the clinical trials and successfully made it to the market are less than 15 amino acids in length, and 20 % of them are synthetic[27, 89].

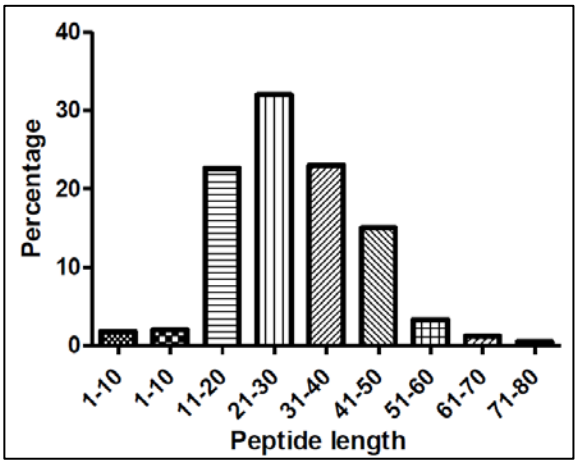

Figure 4: Percentage of peptide length within anti-fungal peptides.

5. Posttranslational modifications of natural AFPs

Several post-translation modifications have been observed in naturally occurring AFPs, and each modification could have an influence on the AFPs activity. The knowledge of these modifications is important to reach a full understanding about the peptide activity and later, to improve the activity or solve problems related to the antifungal activity. The common modifications are: glycosylation, amidation of C-terminus, isomerization which includes diasteromers and enantiomers, halogenation, phosphorylation, hydroxylation and cyclization[90]. The data in this section are general and subjected to change at any time, as new AMPs could be published or added to the database. Furthermore, some of the AMPs have not yet been tested for antifungal activity.

5.1. Glycosylation

Generally, the addition of carbohydrates is one of the most common post-translational modifications of proteins and peptides, which normally observed at asparagine or serine/threonine residues[91]. N-linked and O-linked are the

most common types of glycosylation[92], while S-linked is the rarest[93]. In AMPs, glycosylation was observed in proline-rich peptides such as in Drosocin[94] and Pyrrhocoricin[95]. While, the glycosylation of these peptides was essential for full antimicrobial activity, it was not for others , especially the S-linked[96].

Only two glycosylated AFPs are available at AMP database, Datucin[97] and GHH20[98]. The role of glycosylation is not clear in both peptides. Nevertheless, there are couple of examples of O-glycosylated AFPs in the literature; caspofungin[99] and hassallidin A[100]. In both peptides the addition of monosaccharide was sufficient to improve the antifungal activity.

5.2. Amidation of Carboxy Terminus

The main role of amidation is to improve the peptide stability in the presence of aminopeptidases[101] and in some cases to increase the anti-fungal activity[102]. Several examples of AFPs with amidated C-terminal were identified in nature such as ctriporin from scorpions[103] and ranacyclin from *Rana esculenta* skin[104]. Moreover, the amidation has also been a common approach to increase the stability in synthetic AFPs[35].

5.3. Isomerization

In general ,the isomerization (Diasteromers and enantiomers) increases the antimicrobial activity and improves the stability of AMPs[105]. Diasteromer is the ability of a peptide to exist in two conformations, cis and *trans.* Diasteromers have been identified in AMPs (Caenopore-5)[106] as well as in AFPs (Cyclo(L-Phe- 4-OH-L-Pro))[107]. Enantiomers, there are a few examples of naturally existed AMPs with D-conformation, such as bombinin H4[108] ,lactocin S[109] and gramicidin A[110]. However, none of them had any antifungal activity. Several AFPs have been synthesized using D-amino acids in an attempt to improve the peptide stability against proteases[60, 61].

5.4. Halogenation

In nature, the most common halogenations are bromination and chlorination, with an ambiguous function. Bromination is mainly occurred in AMPs, precisely in the tryptophan indole ring, while chlorination is the most observed in AFPs. Bromination has been described in different AMPs such as *Hedistin* and in *hagfish* cathelicidins[35, 111]. The role of indole bromination was suggested to reduce the peptide susceptibility to proteolysis via steric modifications[112]. On the other hand , the role of the chlorination in AFPs is unclear as the remove of cloride did not alter the antifungal activity of naturally chlorinated Misgurin (*Misgurnus anguillicaudatus)*[35].

5.5. Phosphorylation

Nature has produced multiple examples of phosphorylated AMPs. Phosphorylation has been found to be essential for AMPs activity. For instance, enkelytin, an antibacterial peptide derived from *proenkephalin A,* has two phosphoserines that are required for the full activity[113]. Prochromacin and chromacin are also examples in which the modifications are mandatory for peptide activity (glycosylation and phosphorylation)[114]. In AFPs, the situation is different, and phosphorylation may not be required for the antifungal activity. Histatin 1 is an example of a phosphorylated AFP where the modification did not have any effect on the peptide activity; however, it did increase the stability of the peptide in saliva[115].

5.6. Hydroxylation

In AMPs, hydroxylation was primarily observed at lysine, arginine, tryptophan and phenylalanine residues. The effect of hydroxylation on the peptides is unclear with contradicted effects. For example, the hydroxylation of the MGD-2 peptide was essential for antimicrobial activity; however, other studies have suggested that the peptide activity did not change by the hydroxylation[116]. The effect of hydroxylation in AFPs is also varied. The Cecropins AMP family is an example of hydroxylated lysine peptides, and only cecropin B has shown anti-fungal activity[117]. Halocyamines, tetrapeptides from *Halocynthia roretzi*, are example of dihydroxyphenylalanine modified AFP[118]. Styelin D and

callinectin are examples of AMPs with dihydroxyarginine and hydroxyl-tryptophan residues, respectively[116, 118], but both peptides did not display fungicidal activity.

5.7. Methylation

The most common sites of methylation are tyrosine and lysine residues. Clavanins are natural AMPs with a methylated tyrosine[119]; however, none of them has exhibited antifungal properties. The synthetic cecropin A-melittin is one of few examples of methylated AMPs that possess antifungal activity. It has been shown that the methylation decreased the hemolytic activity and promoted the selectivity against certain microorganisms, but it did not increase the antifungal activity[120].

5.8. Cyclization

Although is not considered a posttranslational modification, cyclization unique properties to the peptides, such as increasing the antifungal activity, reducing the toxicity and improving the stability in proteases[121].Tunicyclins B[24, 122] and cyclopsychotride A[123] are examples of cyclic naturally occurring AFPs. Moreover, there are several examples of synthetic antifungal peptides such as Datomycin, where the cyclization has improved the antifungal activity[124].

6. Disadvantages of AMPs and AFPs, and Proposed Solutions

The general disadvantages of AMPs, and AFPs specifically, could be summarized as: poor oral and tissue absorption, rapid in vivo degradation, poor stability (shelf-life) and potential immunogenicity. Moreover, most peptides are rapidly excreted, poorly bioavailable and salt sensitive. Human toxicity and hemolysis have also been observed with some AMPs. These issues will be addressed in detail below.

6.1. Stability

The stability is not an issue for naturally occurring AFPs within their environment for multiple reasons. First, most of the AFPs are derived by proteolysis of larger proteins or peptides; therefore, equilibrium exists between peptide generation and degradation. For example, buforin II is generated from histone 2A[125], lactoferricin from lactoferrin[126] and histatin-5 from histatin-1. Second, small peptide fragments that are generated via proteolysis of the active peptide, in some case, maintain some antifungal activity. For example, 12 fragments of histatin 1 have been identified and most of them retain antifungal activity. Finally, there might be something within the physiological environment that supports peptide stability and increases the half-life. For instance, the ability of histatin-1 to bind to hydroxyapatite within the enamel pellicle decreases the proteolytic degradation, and consequently increases the half-life[115].

For synthetic peptides, stability and bioavailability problems may be solved via peptide formulations or modifications. Several approaches have been used to improve the stability, such as liposome-encapsulation[127], use of peptoids[128], D-conformation-based peptide[129], and β-peptides[130]. The use of different peptide formulations has been the major approach to improve peptide bioavalability, delivery, and stability. The melittin-lipid disk is an example of a formulated peptide in which polyethylene glycol-stabilized lipid was fused to the melittin peptide to reduce toxicity and improve bioavailability[131]. Carbon nanotubes and magnetics nanoparticles are also useful tools for drug delivery; therefore, this may represent a promising avenue of research for peptide delivery[132].

6.2. Specificity and Toxicity

One major challenge in designing new antifungal drug is achieving high specificity toward fungal cells. The perfect antifungal drug would ideally have an affinity for multiple targets within the fungi, and the targets must be accessible and relatively immutable. In therapeutics, drug toxicity is directly proportional to the concentration; however, the toxic concentration varies between the drugs. Host cell toxicity would be solved by increasing the specificity which required a full understanding of the mechanism by which the peptides recognize their target[42,81].

Natural AFPs, within their tissues, have shown a high target specificity and low toxicity. Most multicellular organisms express a cocktail of peptides within their `defensive' tissues, in which the cocktail contains several classes of AMPs and AFPs[29]. Furthermore, these peptides probably have a synergistic effect and work at low concentrations far from their toxic levels[133]. The synergistic effect is absent in most in vitro assays, since each AFP is tested individually. Furthermore, different isoforms of the same peptide could be present at the same time. For example, different forms of *Rhesus* θ defensins (RTD-1, RTD-2, and RTD-3) have been identified in leukocytes of *Rhesus macaques*, where the cellular abundance of the three peptides (RTD1, RTD-2 and RTD-3) is differ at a ratio of 29:1:2, respectively. In spite of having multiple forms, all of the RTD isoforms have the same antifungal activity; however, they do display distinct antibacterial activity as well as differences in the net charges RTD-1 (+5), RTD-2 (+6), and RTD-3 (+4)[124].

Table 4. Salt-resistant peptides. Most of this data were generated using antimicrobial data base until the writing of this article

Name	Sequence	Source	Secondary structure	Disulfide bond	Activity (MIC)	Net charge	Ref.
Thanatin Thanatin-1 S-Thanatin	GSKKPVPIIYCNRRTGKCQRM GSKKPVPIIYCNRRGKCQRM GSKKPVPIIYCNRRSGKCQRM	*Podisus maculiventris*	beta	C11-C18	*-N. crassa* 0.6-1.2 µM *-B. cinerea* 1.2-2.5 µM *-N. haematococca* 1.2-2.5 µM *-T. viride* 1.2-2.5 µM *-A. brassicola* 2.5-5 µM *-F. culmorumn* 2.5-5 µM *-A. pisi* 5-10 µM *-F. oxysporumn* 10-20 µM *- C. albicans* 25-50 µM	+6	177
RTD-1 RTD-2 RTD-3	GFCRCLCRRGVCRCICTR GVCRCLCRRGVCRCLCRR GFCRCICRRGFCRCICTR	*Rhesus Macaque*	beta	C3-C16 C5-C14 C7-C12	*-C.albicans* 1µg/ml *- C.neoformans* 4µg/ml	+5 +6 +4	139 124
Tachyplesin I Tachyplesin II	KWCFRVCYRGICYRRCR RWCFRVCYRGICYRKCR	*Tachypleus tridentatus*	beta	C3-C16 C7-C12	*-C. albicans* 3.1 µg/ml *-C. neoformans* 1.56 µg/ml *-C. kefyr* 0.9 µM *-C. tropicalis* 0.5 µM	+6	76 140
Arenicin-1	RWCVYAYVRVRGVLVRYRRCW	*Arenicola marina*	beta	C3-C20	*-C. albicans* 4.5 µg/ml	+6	141
Protegrin 1	RGGRLCYCRRRFCVCVGR	*Pig*	beta	C6-C15 C8-C13	*-C. neoformans* 2 µM *-C. albicans* 4 µM	+6	178
Ci-MAM-A24	WRSLGRTLLRLSHALKPLARRSGW	*Ciona intestinalis*	helix	no	*-C. albicans* 6 µM	+6	142
N-[RLLR]2-*C*	RLLRRLLR	*synthetic*	helix	no	*-C. albicans* 0.5 µg/ml *-S. cerevisia* 0.5 µg/ml *-C. neoformans* 0.5 µg/ml	+4	179
P-18	KWKLFKKIPKFLHLAKKF	*synthetic*	helix	no	*-C. albicans* 2-4 µM	+7	159

DCD-1	SSLLEKGLDGAKKAVGGLGKL GKDAVEDLESVGKGAVHDVK DVLDSV	*Homo sapiens*	helix	no	-*C. albicans* 10 μg /ml	-2	143, 180
Melittin	GIGAVLKVLTTGLPALISWIKR KRQQ	*Apis mellifera*	helix	no	-*C. albicans* NA	+5	148
Pelteobagrin	GKLNLFLSRLEILKLFVGAL	*Yellow catfish*	unknown	no	-*C. albicans* 5.4 μM	+2	181

The literature has provided several examples in which the cytotoxicity of synthetic peptides was reduced successfully via modifications in the peptide composition[134-136]. However, each case is unique and the solutions varied. An example of reduced toxicity was observed with the melittin peptide, where cytotoxicity was reduced by fusing melittin with cecropin A or the magainin peptide rather than by amino acids substitution[137, 138].

6.3. Salt sensitivity

Salt sensitivity may present the greatest challenge for the majority of AMPs as and AFPs for clinical uses as the activity will be inhibited at physiological concentration of ions. Table 4 shows synthetic and natural AFPs that seem to maintain an activity at the presence of salts. Since salt insensitivity is only found in a small number of peptides, the general biochemical properties of these peptides will be discusses briefly.

One parameter is the *net charge* that varies dramatically between anionic and cationic peptides; however, the majority of salt insensitive peptides are cationic with a net charge greater or equal to +4 at pH 7. The amphipathicity has been observed in this group with an exception of RTD-1 which did not display any amphipathicity, although the surface models suggested a clustering of positive charges[77].

The second parameter of salt-insensitive peptide is the *secondary structure.* This group can be subdivided into two groups, helices and sheets, where each group has something in common other than secondary structure. By looking to the amino acid composition, most β-structure peptides have more arginine than lysine, and also share the ability to form disulfide bonds. Studying the peptide structure and amino acid compositions of β- peptides sheds light on the role of disulfide bonds and cyclization in salt tolerance. It has been found the cyclization and disulfide bonds are essential for the salt insensitivity in RTD-1[139], TP-1[140], and arenicin-1[141] peptides. However, the essential factor in potegrin 1 was structure rigidity and the presence of disulfide bonds did not have any effect[75]. The major general conclusion is from looking at salt-insensitive β-peptides is that disulfide bonds and/or structure rigidity is crucial to salt insensitivity.

On the contrary, α-helical peptides did not have any cysteine nor disulfide bonds. Peptides in α-helical subclass did not show any common essential feature. The only observed similarity is that the majority of α-helices have lysine more than arginine in their sequence. For instance, it has been reported that the substitution of arginine to lysine in Ci-MAM-A24 increased salt sensitivity[142]. This fact cannot be generalized as in some cases the increase in arginine percentages led to increase salt sensitivity[125]. DCD-1 is a unique example of α-helical peptide, that has been shown to be only 20% active at low salt conditions and the activity is retained by increasing the salt content[143]. The common factor in all salt resistant peptides is that the fungicidal activity is accomplished via pore formation or membrane permeabilization.

7. Conclusions

Antifungal peptides are excellent models for drug discovery exhibiting unique characteristics such as low level of resistance reaching the absent, high specificity, broad spectrum, and unique mode of action. Despite the distinctiveness, only few examples of antifungal peptides have successfully reached the market. The biophysical characteristics and mode of action in antifungal peptides are diverse to a limit that probably each peptide family has its own structure and structure activity relationship

(SAR). This review establishes the bases gathering all possible information including the biophysical characteristics of antifungal peptides available in "AMP database" regardless the source, the net charge, the secondary structure, and the mode of action. The best path for a drug utilizing AFP model to treat systematic yeast infection should be started from the few salt resistant peptides to expand the site of action, and then extensively study the structure activity relationship to improve the activity, and finally utilize the natural posttranslation modification to improve the activity and increase the stability.

REFERENCES

(1) Law, D., Moore, C. B., Wardle, H. M., Ganguli, L. A., Keaney, M. G. L. and Denning, D. W. High prevalence of antifungal resistance in candida spp from patients with aids, *Journal of Antimicrobial Chemotherapy*; 1994; 34: 659-668.

(2) Kanafani, Z. A. and Perfect, J. R. Resistance to antifungal agents: Mechanisms and clinical impact, *Clinical Infectious Diseases*, 2008; 46: 120-128.

(3) Tobudic, S., Kratzer, C. and Presterl, E. Azole-resistant Candida spp. - emerging pathogens?, *Mycoses*, 2012; 55: 24-32.

(4) Pfaller, M. A. Antifungal Drug Resistance: Mechanisms, Epidemiology, and Consequences for Treatment, *American Journal of Medicine,* 2012; 125: S3-S13.

(5) Hitchcock, C. A., Russell, N. J. and Barrettbee, K. J. Sterols in candida-albicans mutants resistant to polyene or azole antifungals, and of a double mutant candida-albicans 6.4, *Crc Critical Reviews in Microbiology*, 1987; 15: 111-115.

(6) Dick, J. D., Merz, W. G. and Saral, R. Incidence of polyene-resistant yeasts recovered from clinical specimens, *Antimicrobial Agents and Chemotherapy*, 1980; 18: 158-163.

(7) Law, D., Moore, C. B. and Denning, D.W. Amphotericin B resistance testing of Candida spp.: a comparison of methods, *Journal of Antimicrobial Chemotherapy*, 1997; 40: 109-112.

(8) Young, L. Y., Hull, C. M. and Heitman, J. Disruption of ergosterol biosynthesis confers resistance to amphotericin B in Candida lusitaniae, *Antimicrobial Agents and Chemotherapy*, 2003; 47, 2717-2724.

(9) Krogh-Madsen, M., Arendrup, M. C., Heslet, L. and Knudsen, J. D. Amphotericin B and caspofungin resistance in Candida glabrata isolates recovered from a critically ill patient, *Clinical Infectious Diseases*, 2006; 42: 938-944.

(10) Drutz, D. J. and Lehrer, R. I. Development of amphotericin b-resistant candida-tropicalis in a patient with defective leukocyte function, *American Journal of the Medical Sciences*, 1978; 276: 77-92.

(11) Laverdiere, M., Lalonde, R. G., Baril, J. G., Sheppard, D. C., Park, S. and Perlin, D. S. Progressive loss of echinocandin activity following prolonged use for treatment of Candida albicans oesophagitis, *Journal of Antimicrobial Chemotherapy*, 2006; 57: 705-708.

(12) Hakki, M., Staab, J.F. and Marr, M. A. Emergence of a Candida krusei isolate with reduced susceptibility to caspofungin during therapy, *Antimicrobial Agents and Chemotherapy*, 2006; 50: 2522-2524.

(13) Pfaller, M. A., Diekema, D. J., Andes, D., Arendrup, M. C., Brown, S. D., Lockhart, S. R., Motyl, M., Perlin, D. S. and Testing, C. S. A. Clinical breakpoints for the echinocandins and Candida revisited: Integration of molecular, clinical, and microbiological data to arrive at species-specific interpretive criteria, *Drug Resistance Updates*, 2011; 14: 164-176.

(14) Desnos-Ollivier, M., Moquet, O., Chouaki, T., Guerin, A.-M., and Dromer, F. Development of Echinocandin Resistance in Clavispora lusitaniae during Caspofungin Treatment, *Journal of Clinical Microbiology*, 2011; 49: 2304-2306.

(15) Moudgal, V., Little, T., Boikov, D. and Vazquez, J. A. Multiechinocandin- and multiazole-resistant Candida parapsilosis solates serially obtained during therapy for prosthetic valve endocarditis,

Antimicrobial Agents and Chemotherapy, 2005; 49: 767-769.

(16) Zarrinfar, H., Mirhendi, H., Fata, A., Khodadadi, H. and Kordbacheh, P. Detection of Aspergillus flavus and A. fumigatus in Bronchoalveolar Lavage Specimens of Hematopoietic Stem Cell Transplants and Hematological Malignancies Patients by Real-Time Polymerase Chain Reaction, Nested PCR and Mycological Assays, *Jundishapur J Microbiol*, 2015; 8: e13744.

(17) Ribes, J. A., Vanover-Sams, C. L. and Baker, D. J. Zygomycetes in Human Disease, 2000.

(18) Nucci, M. and Anaissie, E. Fusarium Infections in Immunocompromised Patients, *Clinical Microbiology Review*, 2007; 20: 695-704.

(19) Thornton, C. R., Ryder, L. S., Le Cocq, K. and Soanes, D. M. Identifying the emerging human pathogen Scedosporium prolificans by using a species-specific monoclonal antibody that binds to the melanin biosynthetic enzyme tetrahydroxynaphthalene reductase, *Environ Microbiol*, 2015; 17: 1023-1038.

(20) Kaur, J., Duan, S. Y., Vaas, L. A. I., Penesyan, A., Meyer, W., Paulsen, I. T. and Nevalainen, H. Phenotypic Profiling of Scedosporium aurantiacum, an Opportunistic Pathogen Colonizing Human Lungs, *PLoS One,* 2015; 10.

(21) Zhai, B. and Lin, X. Recent Progress on Antifungal Drug Development, *Current Pharmaceutical Biotechnology*, 2011; 12: 1255-1262.

(22) Woosley, R. L. and Cossman, J. Drug development and the FDA's critical path initiative, *Clinical Pharmacology & Therapeutics*, 2007; 81: 129-133.

(23) Campese, M., Sun, X., Bosch, J. A., Oppenheim, F. G. and Helmerhorst, E. J. Concentration and fate of histatins and acidic proline-rich proteins in the oral environment, *Archives of Oral Biology*, 2009; 54: 345-353.

(24) Tian, J., Shen, Y., Yang, X., Liang, S., Shan, L., Li, H., Liu, R. and Zhang, W. Antifungal Cyclic Peptides from Psammosilene tunicoides, *Journal of Natural Products*, 2010; 73: 1987-1992.

(25) Jose F. Marcos, M. G., Eleonora Harries, Lourdes Carmona, Alberto Muñoz. Antifungal Peptides: Exploiting Non-Lytic Mechanisms and Cell Penetration Properties In *Small Wonders: Peptides for Disease Control* (Kanniah Rajasekaran, J. W. C., Jesse M. Jaynes, Emilio Montesinos, Ed.), 2012; 337-357, American Chemical Society.

(26) de Lucca, A. J. and Walsh, T. J. Antifungal peptides: Novel therapeutic compounds against emerging pathogens, *Antimicrobial Agents and Chemotherapy*, 1999; 43: 1-11.

(27) Vanessa M.S. Duncan, D.A.O.N. Commercialization of antifungal peptides, *Fungal Biology Reviews*, 2013; 26: 156-165.

(28) De Luca, A. J. and Walsh, T. J. Antifungal peptides: Origin, activity, and therapeutic potential, *Rev Iberoam Micol*, 2000; 17: 116-120.

(29) Zasloff, M. Antimicrobial peptides of multicellular organisms, *Nature,* 2002; 415: 389-395.

(30) Tossi, A., Sandri, L. and Giangaspero, A. Amphipathic, alpha-helical antimicrobial peptides, *Biopolymers*, 2000; 55: 4-30.

(31) Shoeib Moradi, S. S., Alireza M. Ansari and Soroush Sardari. Peptidomimetics and their Applications in Antifungal Drug Design, 2009; 327-344, Anti-Infective Agents in Medicinal Chemistry.

(32) Wiesner, J. and Vilcinskas, A. Antimicrobial peptides The ancient arm of the human immune system, *Virulence,* 2010; 1: 440-464.

(33) Rollins-Smith, L. A., Reinert, L. K., O'Leary, C. J., Houston, L. E. and Woodhams, D. C. Antimicrobial peptide defenses in amphibian skin, *Integrative and Comparative Biology*, 2005; 45: 137-142.

(34) Jager, S., Stange, E. F. and Wehkamp, J. 2010 Antimicrobial peptides in gastrointestinal inflammation, *International journal of inflammation*,2010; 910283.

(35) Andreu, D. and Rivas, L. Animal antimicrobial peptides: An overview, *Biopolymers*, 1998; 47: 415-433.

(36) Marshall, S. H. and Arenas, G. Antimicrobial peptides: A natural alternative to chemical antibiotics

and a potential for applied biotechnology, *Electronic Journal of Biotechnology*, 2003; 6: 271-284.

(37) Butu, M. and Butu, A. Antimicrobial peptides - natural antibiotics, *Romanian Biotechnological Letters*, 2011; 16: 6135-6145.

(38) Wang, G., Li, X. and Wang, Z. APD2: the updated antimicrobial peptide database and its application in peptide design, *Nucleic Acids Research*, 2009; 37: D933-D937.

(39) Zhang, Z. and Zhu, S. Functional role of charged residues in drosomycin, a Drosophila antifungal peptide, *Developmental and Comparative Immunology*, 2010; 34: 953-958.

(40) Burrows, L. L., Stark, M., Chan, C., Glukhov, E., Sinnadurai, S. and Deber, C. M. Activity of novel non-amphipathic cationic antimicrobial peptides against Candida species, *Journal of Antimicrobial Chemotherapy*, 2006; 57: 899-907.

(41) Sagaram, U. S., Pandurangi, R., Kaur, J., Smith, T. J. and Shah, D. M. Structure-Activity Determinants in Antifungal Plant Defensins MsDef1 and MtDef4 with Different Modes of Action against Fusarium graminearum, *Plos One,* 2011; 6.

(42) Yeaman, M. R. and Yount, N. Y. Mechanisms of antimicrobial peptide action and resistance, *Pharmacological Reviews*, 2003; 55: 27-55.

(43) Yang, X. W., Lee, W. H. and Zhang, Y. Extremely Abundant Antimicrobial Peptides Existed in the Skins of Nine Kinds of Chinese Odorous Frogs, *Journal of Proteome Research*, 2012; 11: 306-319.

(44) Xiao, Y., Meng, F., Qiu, D. and Yang, X. Two novel antimicrobial peptides purified from the symbiotic bacteria Xenorhabdus budapestensis NMC-10, *Peptides*, 2012; 35: 253-260.

(45) Shimizu, M., Shigeri, Y., Tatsu, Y., Yoshikawa, S. and Yumoto, N. Enhancement of antimicrobial activity of neuropeptide Y by N-terminal truncation, *Antimicrobial Agents and Chemotherapy*, 1998; 42: 2745-2746.

(46) Mechler, A., Praporski, S., Atmuri, K., Boland, M., Separovic, F. and Martin, L. L. Specific and selective peptide-membrane interactions revealed using quartz crystal microbalance, *Biophysical Journal*, 2007; 93: 3907-3916.

(47) Laforce, F. M. and Boose, D. S. Effect of zinc and phosphate on an antibacterial peptide isolated from lung lavage, *Infection and Immunity*, 1984; 45: 692-696.

(48) Brogden, K. A., DeLucca, A. J., Bland, J. and Elliott, S. Isolation of an ovine pulmonary surfactant-associated anionic peptide bactericidal for Pasteurella haemolytica, *Proceedings of the National Academy of Sciences of the United States of America,* 1996; 93: 412-416.

(49) Brogden, K. A., Ackermann, M., McCray, P. B. and Tack, B. F. Antimicrobial peptides in animals and their role in host defences, *International Journal of Antimicrobial Agents*, 2003; 22: 465-478.

(50) Harris, F., Dennison, S. R. and Phoenix, D. A. Anionic Antimicrobial Peptides from Eukaryotic Organisms, *Current Protein & Peptide Science,* 2009; 10: 585-606.

(51) Soscia, S. J., Kirby, J. E., Washicosky, K. J., Tucker, S. M., Ingelsson, M., Hyman, B., Burton, M. A., Goldstein, L. E., Duong, S., Tanzi, R. E. and Moir, R. D. The Alzheimer's Disease-Associated Amyloid beta-Protein Is an Antimicrobial Peptide, *Plos One,* 2010; 5: 10.

(52) Paulmann, M., Arnold, T., Linke, D., Oezdirekcan, S., Kopp, A., Gutsmann, T., Kalbacher, H., Wanke, I., Schuenemann, V. J., Habeck, M., Buerck, J., Ulrich, A. S. and Schittek, B. Structure-Activity Analysis of the Dermcidin-derived Peptide DCD-1L, an Anionic Antimicrobial Peptide Present in Human Sweat, *Journal of Biological Chemistry*, 2012; 287: 8434-8443.

(53) Litovchick, A. and Rando, R. R. Stereospecificity of short Rev-derived peptide interactions with RRE IIB RNA, *Rna-a Publication of the Rna Society*, 2003; 9: 937-948.

(54) Linde, C. M. A., Hoffner, S. E., Refai, E. and Andersson, M. In vitro activity of PR-39, a proline-arginine-rich peptide, against susceptible and multi-drug-resistant Mycobacterium tuberculosis, *Journal*

of Antimicrobial Chemotherapy, 2001; 47: 575-580.

(55) Wade, D., Boman, A., Wahlin, B., Drain, C. M. andreu, D., Boman, H. G. and Merrifield, R. B. All-d amino acid-containing channel-forming antibiotic peptides, *Proceedings of the National Academy of Sciences of the United States of America*, 1990; 87: 4761-4765.

(56) Bland, J. M., De Lucca, A. J., Jacks, T. J. and Vigo, C. B. All-D-cecropin B: Synthesis, conformation, lipopolysaccharide binding, and antibacterial activity, *Molecular and Cellular Biochemistry*, 2001; 218: 105-111.

(57) Elmquist, A. and Langel, U. In vitro uptake and stability study of pVEC and its all-D analog, *Biological Chemistry*, 2003; 384: 387-393.

(58) Chen, Y. X., Vasil, A. I., Rehaume, L., Mant, C. T., Burns, J. L., Vasil, M. L., Hancock, R. E. W. and Hodges, R. S. Comparison of biophysical and biologic properties of alpha-helical enantiomeric antimicrobial peptides, *Chemical Biology & Drug Design*, 2006; 67: 162-173.

(59) Marcos, J. F. and Gandia, M. Antimicrobial peptides: to membranes and beyond, *Expert Opinion on Drug Discovery*, 2009; 4: 659-671.

(60) den Hertog, A. L., Sang, H., Kraayenhof, R., Bolscher, J. G. M., Van't Hof, W., Veerman, E. C. I. and Amerongen, A. V. N. Interactions of histatin 5 and histatin 5-derived peptides with liposome membranes: surface effects, translocation and permeabilization, *Biochemical Journal*, 2004; 379: 665-672.

(61) Ruissen, A. L. A., Groenink, J., Krijtenberg, P., Walgreen-Weterings, E., van 't Hof, W., Veerman, E. C. I. and Amerongen, A. V. N. Internalisation and degradation of histatin 5 by Candida albicans, *Biological Chemistry*, 2003; 384: 183-190.

(62) Wang, Y.-P. and Lai, R. Insect Antimicrobial Peptides: Structures, Properties and Gene Regulation, *Zoological Research*, 2010; 31: 27-34.

(63) Podda, E., Benincasa, M., Pacor, S., Micali, F., Mattiuzzo, M., Gennaro, R. and Scocchi, M. Dual mode of action of Bac7, a proline-rich antibacterial peptide, *Biochimica Et Biophysica Acta-General Subjects*, 2006; 1760: 1732-1740.

(64) Sato, H. and Felix, J. B. Peptide-membrane interactions and mechanisms of membrane destruction by amphipathic alpha-helical antimicrobial peptides, *Biochimica Et Biophysica Acta-Biomembranes*, 2006; 1758: 1245-1256.

(65) Rosengren, K. J., Clark, R. J., Daly, N. L., Goransson, U., Jones, A. and Craik, D. J. Microcin J25 has a threaded sidechain-to-backbone ring structure and not a head-to-tail cyclized backbone, *Journal of the American Chemical Society*, 2003; 125: 12464-12474.

(66) Martinez, B., Suarez, J. E. and Rodriguez, A. Lactococcin 972: A homodimeric lactococcal bacteriocin whose primary target is not the plasma membrane, *Microbiology-Uk*, 1996; 142: 2393-2398.

(67) Lee, S. Y., Lee, B. L. and Soderhall, K. Processing of an antibacterial peptide from hemocyanin of the freshwater crayfish Pacifastacus leniusculus, *Journal of Biological Chemistry*, 2003; 278: 7927-7933.

(68) Iwatsuki, M., Tomoda, H., Uchida, R., Gouda, H., Hirono, S. and Omura, S. Lariatins, antimycobacterial peptides produced by Rhodococcus sp K01-B0171, have a lasso structure, *Journal of the American Chemical Society*, 2006; 128: 7486-7491.

(69) Mor, A., Hani, K. and Nicolas, P. The vertebrate peptide antibiotics dermaseptins have overlapping structural features but target specific microorganisms, *Journal of Biological Chemistry*, 1994; 269: 31635-31641.

(70) Schibli, D. J., Nguyen, L. T., Kernaghan, S. D., Rekdal, O. and Vogel, H. J. Structure-function analysis of tritrpticin analogs: Potential relationships between antimicrobial activities, model membrane interactions, and their micelle-bound NMR structures, *Biophysical Journal*, 2006; 91: 4413-4426.

(71) Cabras, T., Longhi, R., Secundo, F., Nocca, G., Conti, S., Polonelli, I., Fanali, C., Inzitari, R., Petruzzelli, R., Messana, I., Castagnola, M. and

Vitali, A. Structural and functional characterization of the porcine proline-rich antifungal peptide SP-B isolated from salivary gland granules, *Journal of Peptide Science*, 2008; 14: 251-260.

(72) Sitticholke, S. *GCG file managment*, Humana press, New Jersy, USA, 2003.

(73) David, D. J. A. B. *Adrenergic Receptor Protocols*, Vol. 126, Humana press, New jersy,USA, 2000.

(74) Zelezetsky, I. and Tossi, A. Alpha-helical antimicrobial peptides - Using a sequence template to guide structure-activity relationship studies, *Biochimica Et Biophysica Acta-Biomembranes*, 2006; 1758: 1436-1449.

(75) Matejuk, A., Leng, Q., Begum, M. D., Woodle, M. C., Scaria, P., Chou, S. T. and Mixson, A. J. Peptide-based antifungal therapies against emerging infections, *Drugs of the Future,* 2010; 35: 197-217.

(76) Miyata, T., Tokunaga, F., Yoneya, T., Yoshikawa, K., Iwanaga, S., Niwa, M., Takao, T. and Shimonishi, Y. Antimicrobial peptides, isolated from horseshoe-crab hemocytes, tachyplesin-ii, and polyphemusin-i and polyphemusin-ii - chemical structures and biological-activity, *Journal of Biochemistry*, 1989; 106: 663-668.

(77) Trabi, M., Schirra, H. J. and Craik, D. J. Three-dimensional structure of RTD-1, a cyclic antimicrobial defensin from rhesus macaque leukocytes, *Biochemistry*, 2001; 40: 4211-4221.

(78) Helmerhorst, E. J., van't Hof, W., Breeuwer, P., Veerman, E. C. I., Abee, T., Troxler, R. F., Amerongen, A. V. N. and Oppenheim, F. G. Characterization of histatin 5 with respect to amphipathicity, hydrophobicity, and effects on cell and mitochondrial membrane integrity excludes a candidacidal mechanism of pore formation, *Journal of Biological Chemistry*, 2001; 276: 5643-5649.

(79) Wei, S. Y., Wu, J. M., Kuo, Y. Y., Chen, H. L., Yip, B. S., Tzeng, S. R. and Cheng, J. W. Solution structure of a novel tryptophan-rich peptide with bidirectional antimicrobial activity, *Journal of Bacteriology*, 2006; 188: 328-334.

(80) Cabiaux, V., Agerberth, B., Johansson, J., Homble, F., Goormaghtigh, E. and Ruysschaert, J. M. Secondary structure and membrane interaction of pr-39, a pro+arg-rich antibacterial peptide, *European Journal of Biochemistry*, 1994; 224: 1019-1027.

(81) Blondelle, S. E. and Lohner, K. Combinatorial libraries: A tool to design antimicrobial and antifungal peptide analogues having lyric specificities for structure-activity relationship studies, *Biopolymers,* 2000; 55: 74-87.

(82) Jiang, Z., Kullberg, B. J., van der Lee, H., Vasil, A. I., Hale, J. D., Mant, C. T., Hancock, R. E. W., Vasil, M. L., Netea, M. G. and Hodges, R. S. Effects of Hydrophobicity on the Antifungal Activity of alpha-Helical Antimicrobial Peptides, *Chemical Biology & Drug Design*, 2008; 72: 483-495.

(83) Makovitzki, A., Avrahami, D. and Shai, Y. Ultrashort antibacterial and antifungal lipopeptides, *Proceedings of the National Academy of Sciences of the United States of America,* 2006; 103: 15997-16002.

(84) Schibli, D. J., Epand, R. F., Vogel, H. J. and Epand, R. M. Tryptophan-rich antimicrobial peptides: comparative properties and membrane interactions, *Biochemistry and Cell Biology-Biochimie Et Biologie Cellulaire,* 2002; 80: 667-677.

(85) Rothstein, D. M., Spacciapoli, P., Tran, L. T., Xu, T., Roberts, F. D., Dalla Serra, M., Buxton, D. K., Oppenheim, F. G. and Friden, P. Anticandida activity is retained in P-113, a 12-amino-acid fragment of histatin 5, *Antimicrobial Agents and Chemotherapy*, 2001; 45: 1367-1373.

(86) Galay, R. L., Maeda, H., Aung, K. M., Umemiya-Shirafuji, R., Xuan, X., Igarashi, I., Tsuji, N., Tanaka, T. and Fujisaki, K. Anti-babesial activity of a potent peptide fragment derived from longicin of Haemaphysalis longicornis, *Tropical Animal Health and Production*, 2012; 44: 343-348.

(87) Nagant, C., Pitts, B., Nazmi, K., Vandenbranden, M., Bolscher, J. G., Stewart, P. S. and Dehaye, J. P. Identification of Peptides Derived from the Human Antimicrobial Peptide LL-37 Active against Biofilms Formed by Pseudomonas aeruginosa Using a Library

of Truncated Fragments, *Antimicrobial Agents and Chemotherapy*, 2012; 56: 5698-5708.

(88) Soliman, W., Wang, L., Bhattacharjee, S. and Kaurt, K. Structure-Activity Relationships of an Antimicrobial Peptide Plantaricin S from Two-Peptide Class Ilb Bacteriocins, *Journal of Medicinal Chemistry*, 2011; 54: 2399-2408.

(89) Fox, J. L. Antimicrobial peptides stage a comeback, *Nature Biotechnology*, 2013; 31, 379-382.

(90) Wang, G. Post-translational Modifications of Natural Antimicrobial Peptides and Strategies for Peptide Engineering, *Curr Biotechnol*, 2014; 1: 72-79.

(91) Gilmore, R. Structural biology: Porthole to catalysis, *Nature,* 2011; 474: 292-293.

(92) Nothaft, H. and Szymanski, C. M. Protein glycosylation in bacteria: sweeter than ever, *Nature Reviews Microbiology*, 2010; 8: 765-778.

(93) Garavelli, J. S. The RESID database of protein modifications as a resource and annotation tool, *Proteomics*, 2004; 4: 1527-1533.

(94) McManus, A. M., Otvos, L., Hoffmann, R. and Craik, D. J. Conformational studies by NMR of the antimicrobial peptide, drosocin, and its non-glycosylated derivative: Effects of glycosylation on solution conformation, *Biochemistry*, 1999; 38: 705-714.

(95) Cociancich, S., Dupont, A., Hegy, G., Lanot, R., Holder, F., Hetru, C., Hoffmann, J. A. and Bulet, P. Novel inducible antibacterial peptides from a hemipteran insect, the sap-sucking bug pyrrhocoris-apterus, *Biochemical Journal,* 1994; 300: 567-575.

(96) Oman, T. J., Boettcher, J. M., Wang, H., Okalibe, X. N. and van der Donk, W. A. Sublancin is not a lantibiotic but an S-linked glycopeptide, *Nature Chemical Biology*, 2011; 7: 78-80.

(97) Mandal, S. M. A novel hydroxyproline rich glycopeptide from pericarp of Datura stramonium: proficiently eradicate the biofilm of antifungals resistant Candida albicans, *Biopolymers*, 2012; 98: 332-337.

(98) Rydengard, V., Shannon, O., Lundqvist, K., Kacprzyk, L., Chalupka, A., Olsson, A.-K., Morgelin, M., Jahnen-Dechent, W., Malmsten, M. and Schmidtchen, A. Histidine-rich glycoprotein protects from systemic Candida infection, *Plos Pathogens,* 2008; 4.

(99) Guo, J., Hu, H., Zhao, Q., Wang, T., Zou, Y., Yu, S., Wu, Q. and Guo, Z. Synthesis and Antifungal Activities of Glycosylated Derivatives of the Cyclic Peptide Fungicide Caspofungin, *Chemmedchem,* 2012; 7: 1496-1503.

(100) Neuhof, T., Seibold, M., Thewes, S., Laue, M., Han, C. O., Hube, B. and von Dohren, H. Comparison of susceptibility and transcription profile of the new antifungal hassallidin A with caspofungin, *Biochem Biophys Res Commun,* 2006; 349: 740-749.

(101) Nell, M. J., Tjabringa, G. S., Wafelman, A. R., Verrijk, R., Hiemstra, P. S., Drijfhout, J. W. and Grote, J. J. Development of novel LL-37 derived antimicrobial peptides with LIPS and LTA neutralizing and antimicrobial activities for therapeutic application, *Peptides*, 2006; 27: 649-660.

(102) Cao, W., Zhou, Y. X., Ma, Y. S., Luo, Q. P. and Wei, D. Z. Expression and purification of antimicrobial peptide adenoregulin with C-amidated terminus in Escherichia coli, *Protein Expression and Purification*, 2005; 40: 404-410.

(103) Fan, Z., Cao, L., He, Y., Hu, J., Di, Z., Wu, Y., Li, W. and Cao, Z. Ctriporin, a New Anti-Methicillin-Resistant Staphylococcus aureus Peptide from the Venom of the Scorpion Chaerilus tricostatus, *Antimicrobial Agents and Chemotherapy*, 2011; 55: 5220-5229.

(104) Mangoni, M. L., Papo, N., Mignogna, G., Andreu, D., Shai, Y., Barra, D. and Simmaco, M. Ranacyclins, a new family of short cyclic antimicrobial peptides: Biological function, mode of action, and parameters involved in target specificity, *Biochemistry*, 2003; 42: 14023-14035.

(105) Pag, U., Oedenkoven, M., Papo, N., Oren, Z., Shai, Y. and Sahl, H. G. In vitro activity and mode of action of diastereomeric antimicrobial peptides against bacterial clinical isolates, *Journal of Antimicrobial Chemotherapy*, 2004; 53: 230-239.

(106) Mysliwy, J., Dingley, A. J., Stanisak, M., Jung, S., Lorenzen, I., Roeder, T., Leippe, M. and Groetzinger, J. Caenopore-5: The three-dimensional structure of an antimicrobial protein from Caenorhabditis elegans, *Developmental and Comparative Immunology*, 2010; 34: 323-330.

(107) Strom, K., Sjogren, J., Broberg, A. and Schnurer, J. Lactobacillus plantarum MiLAB 393 produces the antifungal cyclic dipeptides cyclo(L-Phe-L-Pro and cyclo(L-Phe-trans-4-OH-L-Pro and 3-phenyllactic acid, *Applied and Environmental Microbiology*, 2002; 68:4322-4327.

(108) Mignogna, G., Simmaco, M., Kreil, G. and Barra, D. Antibacterial and hemolytic peptides containing d-alloisoleucine from the skin of bombina-variegata, *Embo Journal*, 1993; 12: 4829-4832.

(109) Mortvedt, C. I., Nissenmeyer, J., Sletten, K. and Nes, I. F. Purification and amino-acid-sequence of lactocin-s, a bacteriocin produced by lactobacillus-sake-l45, *Applied and Environmental Microbiology*, 1991; 57: 1829-1834.

(110) Dubos, R. J. and Cattaneo, C. Studies on a bactericidal agent extracted from a soil bacillus : iii. preparation and activity of a protein-free fraction, *The Journal of experimental medicine,* 1939; 70: 249-256.

(111) Tasiemski, A., Schikorski, D., Le Marrec-Croq, F., Camp, C. P.-V., Boidin-Wichlacz, U. and Sautiere, P.-E. Hedistin: A novel antimicrobial peptide containing bromotryptophan constitutively the marine annelid, expressed in the NK cells-like of Nereis diversicolor, *Developmental and Comparative Immunology,* 2007; 31: 749-762.

(112) Shinnar, A. E., Butler, K. L. and Park, H. J. Cathelicidin family of antimicrobial peptides: proteolytic processing and protease resistance, *Bioorganic Chemistry,* 2003; 31: 425-436.

(113) Goumon, Y., Strub, J. M., Moniatte, M., Nullans, G., Poteur, L., Hubert, P., VanDorsselaer, A., Aunis, D. and MetzBoutigue, M. H. The C-terminal bisphosphorylated proenkephalin-A-(209-237 - peptide from adrenal medullary chromaffin granules possesses antibacterial activity, *European Journal of Biochemistry,* 1996; 235: 516-525.

(114) Strub, J. M., Goumon, Y., Lugardon, K., Capon, C., Lopez, M., Moniatte, M., VanDorsselaer, A., Aunis, D. and MetzBoutigue, M. H. Antibacterial activity of glycosylated and phosphorylated chromogranin A-derived peptide 173-194 from bovine adrenal medullary chromaffin granules, *Journal of Biological Chemistry,* 1996; 271: 28533-28540.

(115) McDonald, E. E., Goldberg, H. A., Tabbara, N., Mendes, F. M. and Siqueira, W. L. Histatin 1 Resists Proteolytic Degradation when Adsorbed to Hydroxyapatite, *Journal of Dental Research*, 2011; 90: 268-272.

(116) Noga, E. J., Stone, K. L., Wood, A., Gordon, W. L. and Robinette, D. Primary structure and cellular localization of callinectin, an antimicrobial peptide from the blue crab, *Developmental and Comparative Immunology*, 2011; 35: 409-415.

(117) Qu, X. M., Steiner, H., Engstrom, A., Bennich, H. and Boman, H. G. Insect immunity - isolation and structure of cecropin-b and cecropin-d from pupae of the chinese oak silk moth, antheraea-pernyi, *European Journal of Biochemistry,* 1982; 127: 219-224.

(118) Taylor, S. W., Craig, A. G., Fischer, W. H., Park, M. and Lehrer, R. I. Styelin D, an extensively modified antimicrobial peptide from ascidian hemocytes, *Journal of Biological Chemistry,* 2000; 275: 38417-38426.

(119) Lee, I. H., Zhao, C. Q., Cho, Y., Harwig, S. S. L., Cooper, E. L. and Lehrer, R. I. Clavanins, alpha-helical antimicrobial peptides from tunicate hemocytes, *Febs Letters,* 1997; 400: 158-162.

(120) Teixeira, V., Feio, M. J., Rivas, L., De la Torre, B. G., Andreu, D., Coutinho, A. and Bastos, M. Influence of Lysine N-epsilon-Trimethylation and Lipid Composition on the Membrane Activity of the Cecropin A-Melittin Hybrid Peptide CA(1-7)M(2-9), *Journal of Physical Chemistry B*, 2010; 114: 16198-16208.

(121) Li, P. and Roller, P. P. Cyclization strategies in

peptide derived drug design, *Current topics in medicinal chemistry*, 2002; 2: 325-341.

(122) Daly, N. L., Koltay, A., Gustafson, K. R., Boyd, M. R., Casas-Finet, J. R. and Craik, D. J. Solution structure by NMR of circulin A: A macrocyclic knotted peptide having anti-HIV activity, *Journal of Molecular Biology*, 1999; 285: 333-345.

(123) Witherup, K. M., Bogusky, M. J., Anderson, P. S., Ramjit, H., Ransom, R. W., Wood, T. and Sardana, M. Cyclopsychotride-a, a biologically-active, 31-residue cyclic peptide isolated from psychotria-longipes, *Journal of Natural Products-Lloydia,* 1994; 57: 1619-1625.

(124) Tran, D., Tran, P. A., Tang, Y. Q., Yuan, J., Cole, T. and Selsted, M. E. Homodimeric theta-defensins from Rhesus macaque leukocytes - Isolation, synthesis, antimicrobial activities, and bacterial binding properties of the cyclic peptides, *Journal of Biological Chemistry,* 2002; 277: 3079-3084.

(125) Kim, H. S., Yoon, H., Minn, I., Park, C. B., Lee, W. T., Zasloff, M. and Kim, S. C. Pepsin-mediated processing of the cytoplasmic histone H2A to strong antimicrobial peptide buforin I, *Journal of Immunology,* 2000; 165: 3268-3274.

(126) Roseanu, A., Florian, P., Condei, M., Cristea, D. and Damian, M. Antibacterial activity of Lactoferrin and Lactoferricin against oral Streptococci, *Romanian Biotechnological Letters,* 2010; 15: 5788-5792.

(127) Malheiros, P. d. S., Daroit, D. J. and Brandelli, A. Food applications of liposome-encapsulated antimicrobial peptides, *Trends in Food Science & Technology*, 2010; 21: 284-292.

(128) Chongsiriwatana, N. P., Miller, T. M., Wetzler, M., Vakulenko, S., Karlsson, A. J., Palecek, S. P., Mobashery, S. and Barron, A. E. Short Alkylated Peptoid Mimics of Antimicrobial Lipopeptides, *Antimicrobial Agents and Chemotherapy*, 2011; 55: 417-420.

(129) Sajjan, U. S., Tran, L. T., Sole, N., Rovaldi, C., Akiyama, A., Friden, P. M., Forstner, J. F. and Rothstein, D. M. P-113D, an antimicrobial peptide active against Pseudomonas aeruginosa, retains activity in the presence of sputum from cystic fibrosis patients, *Antimicrobial Agents and Chemotherapy*, 2001; 45: 3437-3444.

(130) Porter, E. A., Weisblum, B. and Gellman, S. H. Mimicry of host-defense peptides by unnatural oligomers: Antimicrobial beta-peptides, *Journal of the American Chemical Society*, 2002; 124: 7324-7330.

(131) Zetterberg, M. M., Reijmar, K., Pranting, M., Engstrom, A., Andersson, D. I. and Edwards, K. PEG-stabilized lipid disks as carriers for amphiphilic antimicrobial peptides, *Journal of Controlled Release,* 2011; 156: 323-328.

(132) Brandelli, A. Nanostructures as Promising Tools for Delivery of Antimicrobial Peptides, *Mini-Reviews in Medicinal Chemistry*, 2012; 12: 731-741.

(133) Nagaoka, I., Hirota, S., Yomogida, S., Ohwada, A. and Hirata, M. Synergistic actions of antibacterial neutrophil defensins and cathelicidins, *Inflammation Research*, 2000; 49: 73-79.

(134) Pacor, S., Giangaspero, A., Bacac, M., Sava, G. and Tossi, A. Analysis of the cytotoxicity of synthetic antimicrobial peptides on mouse leucocytes: implications for systemic use, *Journal of Antimicrobial Chemotherapy*, 2002; 50: 339-348.

(135) Dawson, R. M., McAllister, J. and Liu, C.-Q. Characterisation and evaluation of synthetic antimicrobial peptides against Bacillus globigii, Bacillus anthracis and Burkholderia thailandensis, *International Journal of Antimicrobial Agents*, 2010; 36: 359-363.

(136) Fjell, C. D., Hiss, J. A., Hancock, R. E. W. and Schneider, G. Designing antimicrobial peptides: form follows function, *Nature Reviews Drug Discovery*, 2012; 11: 37-51.

(137) Ferre, R., Melo, M. N., Correia, A. D., Feliu, L., Bardaji, E., Planas, M. and Castanho, M. Synergistic Effects of the Membrane Actions of Cecropin-Melittin Antimicrobial Hybrid Peptide BP100, *Biophysical Journal*, 2009; 96: 1815-1827.

(138) Klocek, G., Schulthess, T., Shai, Y. and Seelig, J. Thermodynamics of Melittin Binding to Lipid

Bilayers. Aggregation and Pore Formation, *Biochemistry,* 2009; 48: 2586-2596.

(139) Tam, J. P., Lu, Y. A. and Yang, J. L. Correlations of cationic charges with salt sensitivity and microbial specificity of cystine-stabilized beta-strand antimicrobial peptides, *Journal of Biological Chemistry*, 2002; 277: 50450-50456.

(140) Tam, J. P., Lu, Y. A. and Yang, J. L. Marked increase in membranolytic selectivity of novel cyclic tachyplesins constrained with an antiparallel two-beta strand cystine knot framework, *Biochemical and Biophysical Research Communications*, 2000; 267: 783-790.

(141) Ovchinnikova, T. V., Aleshina, G. M., Balandin, S. V., Krasnosdembskaya, A. D., Markelov, M. L., Frolova, E. I., Leonova, Y. F., Tagaev, A. A., Krasnodembsky, E. G. and Kokryakov, V. N. Purification and primary structure of two isoforms of arenicin, a novel antimicrobial peptide from marine polychaeta Arenicola marina, *Febs Letters*, 2004; 577: 209-214.

(142) Fedders, H., Michalek, M., Groetzinger, J. and Leippe, M. An exceptional salt-tolerant antimicrobial peptide derived from a novel gene family of haemocytes of the marine invertebrate Ciona intestinalis, *Biochemical Journal*, 2008; 416: 65-75.

(143) Schittek, B., Hipfel, R., Sauer, B., Bauer, J., Kalbacher, H., Stevanovic, S., Schirle, M., Schroeder, K., Blin, N., Meier, F., Rassner, G. and Garbe, C. Dermcidin: a novel human antibiotic peptide secreted by sweat glands, *Nature Immunology*, 2001; 2: 1133-1137.

(144) Lamberty, M., Caille, A., Landon, C., Tassin-Moindrot, S., Hetru, C., Bulet, P. and Vovelle, F. Solution structures of the antifungal heliomicin and a selected variant with both antibacterial and antifungal activities, *Biochemistry*, 2001; 40: 11995-12003.

(145) Levashina, E. A., Ohresser, S., Bulet, P., Reichhart, J. M., Hetru, C. and Hoffmann, J. A. Metchnikowin, a novel immune-inducible proline-rich peptide from drosophila with antibacterial and antifungal properties, *European Journal of Biochemistry*, 1995; 233: 694-700.

(146) Lai, R., Zheng, Y. T., Shen, J. H., Liu, G. J., Liu, H., Lee, W. H., Tang, S. Z. and Zhang, Y. Antimicrobial peptides from skin secretions of Chinese red belly toad Bombina maxima, *Peptides,* 2002; 23: 427-435.

(147) Goraya, J., Knoop, F. C. and Conlon, J. M. Ranatuerins: Antimicrobial peptides isolated from the skin of the American bullfrog, Rana catesbeiana, *Biochemical and Biophysical Research Communications*, 1998; 250: 589-592.

(148) Park, C. and Lee, D. G. Melittin induces apoptotic features in Candida albicans, *Biochemical and Biophysical Research Communications*, 2010; 394: 170-172.

(149) Fujitani, N., Kawabata, S., Osaki, T., Kumaki, Y., Demura, M., Nitta, K. and Kawano, K. Structure of the antimicrobial peptide tachystatin A, *Journal of Biological Chemistry*, 2002; 277: 23651-23657.

(150) Duvick, J. P., Rood, T., Rao, A. G. and Marshak, D. R. Purification and characterization of a novel antimicrobial peptide from maize (zea-mays l) kernels, *Journal of Biological Chemistry*, 1992; 267: 18814-18820.

(151) Hwang, P. M., Zhou, N., Shan, X., Arrowsmith, C. H. and Vogel, H. J. Three-dimensional solution structure of lactoferricin B, an antimicrobial peptide derived from bovine lactoferrin, *Biochemistry*, 1998; 37: 4288-4298.

(152) Camposolivas, R., Bruix, M., Santoro, J., Lacadena, J., Delpozo, A. M., Gavilanes, J. G. and Rico, M. Nmr solution structure of the antifungal protein from aspergillus-giganteus - evidence for cysteine pairing isomerism, *Biochemistry*, 1995; 34: 3009-3021.

(153) Skerlavaj, B., Gennaro, R., Bagella, L., Merluzzi, L., Risso, A. and Zanetti, M. Biological characterization of two novel cathelicidin-derived peptides and identification of structural requirements for their antimicrobial and cell lytic activities, *Journal of Biological Chemistry*, 1996; 271: 28375-28381.

(154) Huttner, K. M., Brezinski-Caliguri, D. J., Mahoney, M. M. and Diamond, G. Antimicrobial peptide

expression is developmentally regulated in the ovine gastrointestinal tract, *Journal of Nutrition*, 1998; 128: 297S-299S.

(155) Park, C. B., Kim, M. S. and Kim, S. C. Novel antimicrobial peptide from Bufo bufo gargarizans, *Biochemical and Biophysical Research Communications*, 1996; 218: 408-413.

(156) Maerki, C., Meuter, S., Liebi, M., Muehlemann, K., Frederick, M. J., Yawalkar, N., Moser, B. and Wolf, M. Potent and Broad-Spectrum Antimicrobial Activity of CXCL14 Suggests an Immediate Role in Skin Infections, *Journal of Immunology*, 2009; 182: 507-514.

(157) Broekman, D. C., Frei, D. M., Gylfason, G. A., Steinarsson, A., Jornvall, H., Agerberth, B., Gudmundsson, G. H. and Maier, V. H. Cod cathelicidin: Isolation of the mature peptide, cleavage site characterisation and developmental expression, *Developmental and Comparative Immunology*, 2011; 35: 296-303.

(158) Mandal, S. M., Migliolo, L., Franco, O. L. and Ghosh, A. K. Identification of an antifungal peptide from Trapa natans fruits with inhibitory effects on Candida tropicalis biofilm formation, *Peptides*, 2011; 32: 1741-1747.

(159) Gun Lee, D., Shin, S. Y., Maeng, C. Y., Jin, Z. Z., Kim, K. L. and Hahm, K. S. Isolation and characterization of a novel antifungal peptide from Aspergillus niger, *Biochemical and biophysical research communications*, 1999; 263: 646-651.

(160) Craik, D. J., Daly, N. L., Bond, T. and Waine, C. Plant cyclotides: A unique family of cyclic and knotted proteins that defines the cyclic cystine knot structural motif, *Journal of Molecular Biology*, 1999; 294: 1327-1336.

(161) Tam, J. P., Lu, Y. A., Yang, J. L. and Chiu, K. W. An unusual structural motif of antimicrobial peptides containing end-to-end macrocycle and cystine-knot disulfides, *Proceedings of the National Academy of Sciences of the United States of America,* 1999; 96; 8913-8918.

(162) Mello, E. O., Ribeiro, S. F. F., Carvalho, A. O., Santos, I. S., Da Cunha, M., Santa-Catarina, C. and Gomes, V. M. Antifungal Activity of PvD1 Defensin Involves Plasma Membrane Permeabilization, Inhibition of Medium Acidification, and Induction of ROS in Fungi Cells, *Current Microbiology*, 2011; 62: 1209-1217.

(163) Games, P. D., dos Santos, I. S., Mello, E. O., Diz, M. S. S., Carvalho, A. O., de Souza, G. A., Da Cunha, M., Vasconcelos, I. M., Ferreira, B. D., and Gomes, V. M. Isolation, characterization and cloning of a cDNA encoding a new antifungal defensin from Phaseolus vulgaris L. seeds, *Peptides*, 2008; 29: 2090-2100.

(164) Rolland, J. L., Abdelouahab, M., Dupont, J., Lefevre, F., Bachere, E. and Romestand, B. Stylicins, a new family of antimicrobial peptides from the Pacific blue shrimp Litopenaeus stylirostris, *Molecular Immunology*, 2010; 47: 1269-1277.

(165) Cytrynska, M., Mak, P., Zdybicka-Barabas, A., Suder, P. and Jakubowicz, T. Purification and characterization of eight peptides from Galleria mellonella immune hemolymph, *Peptides*, 2007; 28: 533-546.

(166) Silva, F. D., Rezende, C. A., Rossi, D. C. P., Esteves, E., Dyszy, F. H., Schreier, S., Gueiros-Filho, F., Campos, C. B., Pires, J. R. and Daffre, S. Structure and Mode of Action of Microplusin, a Copper II-chelating Antimicrobial Peptide from the Cattle Tick Rhipicephalus (Boophilus) microplus, *Journal of Biological Chemistry*, 2009; 284: 34735-34746.

(167) Kamysz, W., Nadolski, P., Kedzia, A., Cirioni, O., Barchiesi, F., Giacometti, A., Scalise, G., Lukasiak, J. and Okroj, M. In vitro activity of synthetic antimicrobial peptides against Candida, *Polish Journal of Microbiology*, 2006; 55: 303-307.

(168) Chia, C. S. B., Torres, J., Cooper, M. A., Arkin, I. T. and Bowie, J. H. The orientation of the antibiotic peptide maculatin 1.1 in DMPG and DMPC lipid bilayers. Support for a pore-forming mechanism, *Febs Letters*, 2002; 512: 47-51.

(169) Vouldoukis, I., Shai, Y., Nicolas, P. and Mor, A. Broad spectrum antibiotic activity of skin-PYY, *Febs*

Letters, 1996; 380: 237-240.

(170) Lee, Y. S., Yun, E. K., Jang, W. S., Kim, I., Lee, J. H., Park, S. Y., Ryu, K. S., Seo, S. J., Kim, C. H. and Lee, I. H. Purification, cDNA cloning and expression of an insect defensin from the great wax moth, Galleria mellonella, *Insect Molecular Biology*, 2004; 13: 65-72.

(171) Xu, L., Lal, K. and Pollock, J. J. Histatins 2 and 4 are autoproteolytic degradation products of human parotid-saliva, *Oral Microbiology and Immunology*, 1992; 7: 127-128.

(172) Conlon, J. M., Sonnevend, A., Patel, M., Al-Dhaheri, K., Nielsen, P. F., Kolodziejek, J., Nowotny, N., Iwamuro, S. and Pal, T. A family of brevinin-2 peptides with potent activity against Pseudomonas aeruginosa from the skin of the Hokkaido frog, Rana pirica, *Regulatory Peptides*, 2004; 118: 135-141.

(173) El Karim, I. A., Linden, G. J., Orr, D. F. and Lundy, F. T. Antimicrobial activity of neuropeptides against a range of micro-organisms from skin, oral, respiratory and gastrointestinal tract sites, *Journal of Neuroimmunology*, 2008; 200: 11-16.

(174) de Zelicourt, A., Letousey, P., Thoiron, S., Campion, C., Simoneau, P., Elmorjani, K., Marion, D., Simier, P. and Delavault, P. Ha-DEF1, a sunflower defensin, induces cell death in Orobanche parasitic plants, *Planta,* 2007; 226: 591-600.

(175) Tian, C., Gao, B., Rodriguez, M. d. C., Lanz-Mendoza, H., Ma, B. and Zhu, S. Gene expression, antiparasitic activity, and functional evolution of the drosomycin family, *Molecular Immunology*, 2008; 45: 3909-3916.

(176) Slavokhotova, A. A., Odintsova, T. I., Rogozhin, E. A., Musolyamov, A. K., Andreev, Y. A., Grishin, E. V. and Egorov, T. A. Isolation, molecular cloning and antimicrobial activity of novel defensins from common chickweed (Stellaria media L.) seeds, *Biochimie*, 2011; 93: 450-456.

(177) Fehlbaum, P., Bulet, P., Chernysh, S., Briand, J. P., Roussel, J. P., Letellier, L., Hetru, C. and Hoffmann, J. A. Structure-activity analysis of thanatin, a 21-residue inducible insect defense peptide with sequence homology to frog skin antimicrobial peptides, *Proceedings of the National Academy of Sciences of the United States of America,*1996; 93: 1221-1225.

(178) Benincasa, M., Scocchi, M., Pacor, S., Tossi, A., Nobili, D., Basaglia, G., Busetti, M. and Gennaro, R. Fungicidal activity of five cathelicidin peptides against clinically isolated yeasts, *Journal of Antimicrobial Chemotherapy*, 2006; 58: 950-959.

(179) Park, I. Y., Cho, J. H., Kim, K. S., Kim, Y. B., Kim, M. S. and Kim, S. C. Helix stability confers salt resistance upon helical antimicrobial peptides, *Journal of Biological Chemistry*, 2004; 279: 13896-13901.

(180) Lai, Y. P., Peng, Y. F., Zuo, Y., Li, J., Huang, J., Wang, L. F. and Wu, Z. R. Functional and structural characterization of recombinant dermcidin-1L, a human antimicrobial peptide, *Biochemical and Biophysical Research Communications*, 2005; 328: 243-250.

(181) Su, Y. Isolation and identification of pelteobagrin, a novel antimicrobial peptide from the skin mucus of yellow catfish (Pelteobagrus fulvidraco), *Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology*, 2011; 158: 149-154.

مضادات الفطريات الببتيدية: عصر جديد من العقاقير المضادة للفطريات

***يزن العكام*[1]**

[1] قسم العلوم الصيدلانية، كلية الصيدلة، جامعة اليرموك، إربد، الأردن.

ملخص

وجود عدد محدود من الأدوية المضادة للفطريات والتزايد المستمر للسلالات الفطرية المقاومة لهذه المضادات يحفز على ضرورة تطوير أدوية جديدة للفطريات وتعمل بآليات فريدة. إن مضادات الميكروبات الببتيدية عناصر نشطة تعمل كترسانة طبيعية ضد مسببات الأمراض بما في ذلك الفطريات. في هذا البحث نسلط الضوء على جميع الدراسات التي أجريت حول الخصائص البيوفيزيائية للببتيدات والتي تظهر الصفات المضادة للفطريات الموجودة في قاعدة بيانات الببتيدات المضادات للميكروبات حتى شهر أيلول 2015، والتي تشمل على الشحنة التي تحملها الببتيدات والتوزيع الفراغي والتشكل وغيرها من الخصائص. إن جمع الدراسات التي تتحدث عن هذه الخصائص الببتيدية من الممكن أن يكون مدخلاً لاكتشاف وتطوير مضادات جديدة .كما تتحدث هذه الدراسة عن العقبات والمشاكل التي تمنع الببتيدات من أن تصبح أدوية فاعلة وتحاول إيجاد حلول لها لتصبح مرجعا يمكن استعماله في مجال اكتشاف الأدوية المضادة للفطريات.

الكلمات الدالة: مضادات المبكروبات الببتيدية، مضادات الفطريات، الخصائص البيوفيزيائية للببتيدات.

تاريخ استلام البحث 2014/5/4 وتاريخ قبوله للنشر 2015/9/2.