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Comparative Study of *Bombyx mori* Antimicrobial Peptides (AMPs) Retrieved from APD2 Database

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ABSTRACT:

Antimicrobial peptide (AMPs) are rapidly synthesized and released in the hemolymph of insect upon microbial infections as a humoral immune response. These are ribosomally synthesized natural antibiotics produced by all taxonomic ranks (Microbes, Plants, Animals, Insects etc.). AMP synthesis at commercial level will be possible as an alternative candidate to fight against multidrug resistant organisms by using the model organism silkworm *Bombyx mori*. We found that conserved amino acid pattern was more or less precise. Interaction with lipid bilayer can be facilitated due to pI near 10 of major AMPs. Instability index concludes *in vitro* activity as stable, moderately stable and slightly unstable peptides. Stability at wide range of temperature was found unstable, moderately stable and slightly unstable as per aliphatic index of AMPs. As per GRAVY calculation 90% AMPs were hydrophilic, hence directly interact with head of phospholipid bilayer to facilitate penetration. Cell toxicity, hemolytic as well as antimicrobial activity was found moderate due to % hydrophobicity. As per values of Boman index AMPs were showing limited interaction with other proteins/peptides. As per various bioinformatics tools these were found non-allergic, nontoxic, low or normal half-life in intestine environment, antigenic and non-cell penetrating peptides. This study will be supportive for working with AMPs as an alternative to the antibiotics for better effectiveness against multidrug resistant (MDR) microorganisms.

Keywords: Antimicrobial peptides; (AMPs); *Bombyx mori;* Physico-chemical properties; Sequence homology.

[1] INTRODUCTION

In late 1950s *Staphylococcus aureus* had rang the warning bell in the field of antibiotic therapy by showing drug resistance against penicillin. Many microbes using various mechanisms have been showing drug resistance against commercially available and clinically practiced antibiotics [1].Advances in biotechnology, genomics, biochemistry and bioinformatics were also failed

to innovate new antimicrobials i.e. caused the erosion in pipeline of new antibiotic synthesis [2]. In this context AMPs may be future candidates to fight against the infectious agents due to the better properties.

Broad spectrum *in vitro* and/or *in vivo* antimicrobial activity showing AMPs are smaller candidates, hence can be easily synthesized by solid phase chemistry and are less susceptible to the drug resistant microbes. These AMPs can show effect alone or along with other antibiotics [3]. Better efficiency of AMPs can be obtained by chemical modification of N- or C- terminal ends [4], developing analogues by replacing amino acids with unnatural amino acids [5], restricting length [6], polymeric nano-encapsulation of AMPs [7], modifying amphipathic balance [8], synthesizing dendrimeric peptides [9], and formulating combinations with antibiotics, which will be effective against bacteria and fungi.

AMPs are rapidly synthesized and released in the hemolymph of insect upon microbial infections as a humoral immune response [10]. These are ribosomally synthesized natural antibiotics produced by all taxonomic ranks (Microbes, Plants, Animals, and Insect etc.). Silkworm has been domesticated for silk production as an art and agricultural side business. Economical importance and morphological properties of silkworms attracted basic science researchers to find facts in genetics, physiology and pathology [11]. Silkworm *Bombyx mori* is known to produce AMPs [12-18].

The present study determines the predictions of Antimicrobial peptide (AMP) sequences derived from model organism *Bombyx mori* using bioinformatics tools that will be supportive for working with AMPs as an alternative to the antibiotics for better effectiveness against multidrug resistant (MDR) microorganisms.

[2] MATERIALS AND METHODS

Ten AMPs of *Bombyx mori* were retrieved from the APD2 database. All ten sequences were aligned using ClustalW2 multiple sequence alignment tool providing FASTA format sequences and PHYLIP format option was selected as result output. Multiple alignment result were transfer to BoxShade tool as other input sequence format and RTF_new option were selected for output results. Best four homologous sequences of each AMP were also retrieved from APD2 database. Sequence homology were observed by using ClustalW2 multiple alignment tool and pretty printing as well as shading of multiple aligned files were carried out by BoxShade tool. AMPer a database and automated discovery tool were used for similarity search with known mature and propeptide database AMPs, which were used for classification or grouping of unknown AMP. All AMP sequences were analyzed by using ProtParam tool of ExPASy for determining length, amino acid composition, Isoelectric point, instability index, aliphatic index, GRAVY and *in vivo* half-life. Net charge, % hydrophobicity and Boman index were retrieved from APD database search tool. Cell penetrating ability of multiple AMPs were determined using CellPPD tool with its Support Vector Machine (SVM) values, molecular weights, isoelectric points and net charge. Antigenic peptide prediction tool of Immunomedicine Group was used for prediction of antigenic determinants within AMP. EVALLER™ (also referred to as EVALLER 2) web-tool were used for electronically test (e-Testing) allergic potential of AMP on the basis of amino acid sequence. ToxinPred tool were used for predicting regions in AMP, which contributes in toxicity. Half-life of AMP in the intestine were predicted by using HLP tool. Helical Wheel Projections tool were used for helix wheel diagram prediction. Sequence Annotated by Structure (SAS) tool of EMBL-EBI were used for prediction of secondary structures of AMPs.

[3]. RESULTS AND DISCUSSION

1. Retrieved sequences

Total ten antimicrobial sequences of mulberry silkworm *Bombyx mori* were retrieved from APD2 database (http://aps.unmc.edu/AP/prediction/

prediction_ main.php)**.** Retrieved AMP sequence AP00147 belongs to class BM Moricin and AP00373 to class Moricin 2 [12], whereas other AMPs AP00183, AP00350, AP00359, AP00360, AP00361, AP01974, AP01975 and AP02427 were belongs to class Cecropin B [13], Enbocin [14], Lebocin 1/2, Lebocin 3 [15], Lebocin 4 [16], Silkworm 001, Silkworm 002 [17] and CecropinXJ [18], respectively.

2. Conserved amino acid patterns

All the FASTA format AMP sequences were analysised by ClustalW2 multiple sequence alignment tool (http://www.ebi.ac.uk/Tools/msa/clustalw2/) and results were obtained as PHYLIP format option. Pretty printing and shading of multiple aligned sequences were performed using BoxShade tool (http://www.ch.embnet.org/software/BOX_form.html) by providing PHYLIP format output result. Single amino acid residues as Lysine (K), Alanine (A), Glycine (G), Isoleucine (I) and/or Proline (P) were showed to be conserved with less or more precise manner, while up to certain level various amino acid residues were also showed to be conserved as shown in the figure.1.

3. Sequence homology

Best matching AMPs were retrieved from APD2 database by submitting query sequence by deleting one or two amino acid residues from C-terminus to make the sequence unknown because matching for AMP, which were retrieved from same database, were not performed. These matched complete FASTA format sequences were multiple aligned using ClustalW2 tool and pretty printing as well as shading were performed by BoxShade tool as shown in the figure.2. On the basis of detailed information provided by APD2 database AMP sequences of *Bombyx mori* were showing matching with AMPs isolated from different taxonomic ranks *viz*. Noctuid moth *Heliothis virescens*, silkworm *Spodoptera litura*, tobacco hornworm *Manduca sexta*, Tobacco budworm moth *Heliothis virescens*, giant silk moth *Hyalophora cecropia*, Fall webworm *Hyphantria cunea*, Chinese oak silk moth *Antheraea pernyi*, New Zealand deer *Cervus elaphus*, European fire bug *Pyrrhocoris apterus*, milkweed bug *Oncopeltus fasciatus*, Green Sea urchin *Strongylocentrotus droebachiensis*, Southern bell frog *Litoria aurea* or *Litoria raniformis*, barnyard grass seeds *Echinochloa crusgalli* L., ant *Pachycondyla goeldii*, plant *Viola odorata* (Violaceae), marine snail *Rapana venosa* and vespine wasps *Vespa bicolor*. Producing such type of AMPs is not a monopoly of *Bombyx mori i.e*. AMPs were conserved within the various taxonomic ranks for the protection.

4. AMPer matching

AMPer a database and automated discovery tool (http://marray.cmdr.ubc.ca/cgi-bin/amp.pl?userinseq=1) were used for similarity search with known mature and propeptide database AMPs, which were used for classification or grouping of unknown AMP. It is based on a BLAST search, which is performed between query sequence and corresponding Swiss-Prot proteins. Positive match result will be given, if query sequence passes the conditions like matching with HMMs and feature annotation agrees to at least 90% of its length as well as best matching peptide should be at least 50% identical to the Swiss-Prot proteins [19]. AP00147, AP00183, AP02427, AP00350, AP00359, AP00360, AP00361 and AP00373 peptides were identical with mature peptides of AMPer database. AP00147 and AP00373 were showing blast matching with group 22, hence can be grouped under moricin or virescein. AP00183 and AP02427 were matching with group 139, hence can be classified under bactericidin, cecropin or hyphancin groups. AP00350 were showed similar to group 104, hence may belongs to cecropin-A, hyphancin or moricin. AP00359, AP00360 and AP00361 were matching with group 72, hence comes under lebocin family of mature peptides. Blast E-values of these cent percent matched peptides were different. AMPer mature or propeptides were not matching with AP01974 and AP01975 as shown in the table.1.

5. Analysis of AMPs

ProtParam tool (http://web.expasy.org/protparam/) were used for determination of length, amino acid composition, isoelectric point, instability index, aliphatic index, GRAVY and *in vivo* half-life of AMPs.

5.1 Length

Activity of AMP is based on its length because minimum 7-8 amino acids will be needed to orient in amphipathic structure with hydrophilic and hydrophobic faces on both sides of AMP molecule. As per the barrel-stave model at least 8 amino acid β-sheet AMP and 22 amino acid α-helical AMPs can transverse bacterial plasma membrane [20]. Length of AMP has been also associated with toxicity i.e. longer the AMP higher is the toxicity to Erythrocytes. AMPs are oligopeptides having varying lengths from few to over hundreds of amino acids [21]. In these ten AMPs smallest (18 amino acids) length was observed in AP01975 and longest (42 amino acids) for AP00147 and AP00373 as mentioned in the table.2.

5.2 Amino acid rich peptides:

Out of ten AMPs, AP00147 and AP00373 possess 21.4% lysine residues, AP00350 contains 24.3% alanine, while three AMPs *viz.* AP00359, AP00360 and AP00361 are proline rich possess 25%, 21.9 and 21.9 proline residues respectively as shown in the table 2. These AMPs have been further grouped as Glycine/Proline rich and Alanine/Proline rich. Initially it were isolated from intestine of *Sus scrofa* [22], followed by *D. melanogaster* [23], European sap- sucking bug *Pyrrhocoris apterus*, *Apis mellifer* and *Myrmecia gulosa*. Straight forwarded relation were not observed with its mechanism of action and richness of Proline amino acid. In 1991, it was evidenced that Glycine rich AMPs were longer than others and possess 25 to 50% Glycines. In aqueous environment structures were disordered which will be self ordered upon contact with artificial membrane. Helix having Glycine ridge at one side leads to multimerization using helix-helix interaction within the bacterial membrane [24]. Typical example of tryptophan rich AMP (indolicin) was isolated from neutrophils of *Bos taurus*. These linear AMPs are absence of Disulfide Bridge cannot orients into secondary structures in water, whereas proximity with lipid micelles result into wedge shaped structure. Higher affinity were observed at 25% tryptophan rich AMP with neutral POPC and anionic POPG vesicles. A global cationic amphipathic helical 25% Histidine containing AMPs triggers membrane disruption by parallel orientation with plasma membrane surface [25].

5.3 Isoelectric point

Isoelectric point (pI) is the pH where there is zero net charge on an AMP; hence it will affect the solubility of AMP as it precipitates and also loses its biological functions. Isoelectric points of these AMPs are *viz.* AP01974 (6.74), AP00361 (9.52), AP00359 and AP00360 (9.82), AP01975 (9.99), AP00183 (10.64), AP00350 (10.67), AP02427 (10.71), AP00147 and AP00373 (11.36). Only AP01974 has 6.74 pI which is not near to 10 pI otherwise all other AMPs are within the 9.5 to 11.5 pI range (Table 2). Isoelectric point of majority of AMPs should be near to pH 10 similar to detergents or emulsifying agents like soaps, which assists the mechanism of action for interacting with lipid bilayer of biological membrane [26].

5.4 Instability index

The instability index is the estimate of stability of AMP in a test tube. Instability index <40 is the indication of stability of AMPs [27]. AP00147 and AP00373 are highly stable AMPs out of these ten AMPs due to instability index 8.32, whereas AP00361 (35.12), AP00360 and AP00359 (37.09) moderate stability. AP00350 (42.39), AP02427 (61.88), AP01975 (62.19) and AP00183 (67.27) are slightly unstable (Table 2).

5.5 Aliphatic index

Relative volume occupied towards aliphatic side chains (Alanine, Valine, Isoleucine and Leucine) of AMP is aliphatic index. Aliphatic index plays

positive role in the great thermal stability of globular protein. All of these ten AMPs have positive aliphatic index though values are differing from each other due to composition and distribution of hydrophobic amino acids. AP01974 and AP001975 may be less stable in the wide range temperature due to 41.90 and 48.89 aliphatic indexes respectively. Moderate thermostability can be achieved by AP00359 (70), AP00361 (73.12), AP00360 (82.19), AP00350 (95.14), while somewhat greater stability observed in AP00373 (100), AP02427 (100.27), AP00147 (102.38) and 106 for AP00183 as shown in the table 2. Aliphatic index >70 were showed in almost AMPs and indicates stability for wider range of temperature [28]. Positive aliphatic index indicates enhanced thermostability of globular protein.

5.6 GRAVY

Grand Average of Hydropathy (GRAVY) value of AMP is calculated as the sum of hydropathy values of all the amino acids, divided by the number of amino acid residues in the complete sequence. Positive and negative GRAVY is indication of hydrophobicity and hydrophilicity respectively [29]. Single AMP AP00350 has hydrophobicity with 0.232 GRAVY, while all others are showing hydrophilicity viz. AP00147 (-0.09), AP00183 (-0.13), AP00359 (-0.67), AP00360 (-0.5), AP00361 (-0.82), AP00373 (-0.21), AP01974 (-0.79), AP01975 (-0.38) and AP02427 (-0.243). AMPs showing hydrophilicity may attract towards the hydrophilic head group of phospholipid bilayer and then afterwards will be penetrates using hydrophobic residues of AMPs in the tail or trans-membrane lipid layer of plasma membrane of target cells as shown in the table 2.

5.7 *In vivo* half-life

Time taken to disappear half the amount of protein after its synthesis or entry into the cell is considered as half-life. This prediction is based on "N-end rule" for three model organisms as human reticulocytes, yeast and *E.coli*. According to this rule N-terminal residues of a protein plays significant role for maintaining its stability *in vivo* [30]. Estimated half-life of AMPs in the reticulocytes of human are given in hours as AP00183 (1 hour), AP00359, AP00360 and AP00361 (1.1 hour), AP01975 (1.3 hours), AP00147 and AP00373 (4.4 hours) and AP00350 (>20 hours). These values have significance for catabolic study of AMP in the human body. However, estimated half-life of AMPs for yeast cell are given in hours as AP00183 and AP02427 (2 min.), AP00359, AP00360, AP00361 and AP01975 (3 min.), AP0147, AP00350 and AP00373 (>20 hours). Similarly estimated half-life of AMPs in the *E. coli* are given in hours as AP00183, AP01974 and AP02427 (2 min.), AP01975 (3 min.), AP00147, AP00359, AP00360, AP00361 and AP00373 (>10 hours) and AP00350 half-life is not given by tool as shown in the table 2. Study of half-life in the microorganism is essential during the synthesis of AMP using rDNA technology.

Determination of net charge, % hydrophobicity and Boman index was retrieved from APD2 database (http://aps.unmc.edu/AP/prediction/prediction_main.php) by searching detailed information of each APD ID.

5.8 Net charge

Sum of ionizable amino acid residues at particular pH will give rise to anionic or cationic net surface charge to AMPs. All these ten AMPs were cationic in nature viz. +0.5 (AP01974), +2 (AP00350, AP00361, AP01975), +3 (AP00359, AP00360), +6 (AP00183), +7 (AP02427) and +10 (AP00147, AP00373). This cationicity is due to content of Arginine plus lysine (Acidic or positively charged residues) and Aspartic acid plus Glutamic acid (Basic or negatively charged residues). Ratio of Arginine plus lysine to Aspartic acid plus Glutamic acid is responsible for net charge of AMPs as 1:1 ratio in AP01974 (+0.5), 3:5 in AP00350 and AP00361 (+2), 1:3 in AP01975 (+2), 2:5 in AP00359 and AP00360 (+3), 3:9 in AP00183 (+6), 3:10 in AP02427 (+7) and 1:11 in AP00147 and AP00373 (Table 3). Maximum

AMPs were cationic due to rich amount of lysine, Arginine (acidic amino acids) and rarely aspartic acid or Glutamic acid. Most of AMPs possess +2 to +9 net charge, while naturally isolated have +4 to +6 cationicity. Bacterial and other microbes surface have been negatively charged hence cationic AMPs can easily approach towards the plasma membrane of bacteria [31]. Higher the cationicity greater is the antimicrobial activity but this relation were not 100% percent true because enhanced cationicity beyond the limit may reduce the mechanism of action or increases cytotoxicity.

5.9 Hydrophobicity

Amino acids having non-polar nature were responsible for giving hydrophobicity to the AMPs. AMPs were allowed to partition within the microbial cell membrane is because of hydrophobicity. Percent hydrophobic residues observed in these ten AMPs are as AP01974 (23), AP00359 (28), AP00360 and AP00361 (31), AP00373 (42), AP02427 (43), AP01975 (44), AP00147 and AP00183 (45) and 51 within AP00350 (Table 3). Enhanced hydrophobicity beyond the certain limit will cause serious problem of mammalian cell toxicity and forgiveness of antimicrobial activity [31]. Some time increased hydrophobicity may be responsible for more hemolytic activity and less hydrophobicity for declined antimicrobial activity [32].

5.10 Boman index

According to Boman index for AMPs, there is strong correlation in between index itself, its physicochemical properties and biological functions [33]. Boman index is the ability of AMP to bind with other proteins or peptides. Boman index of these ten AMPs is below to the moderate range viz. AP00147 (1.16 kcal/mol), AP00183 (1.4 kcal/mol), AP00350 (1.47 kcal/mol), AP00359 (1.58 kcal/mol), AP00360 (1.43 kcal/mol), AP00361 (1.93 kcal/mol), AP00373 (1.31 kcal/mol), AP001974 (1.83 kcal/mol), AP01975 (1.92 kcal/mol) and AP02427 (1.45 kcal/mol), hence these can interact with limited number of other peptides or proteins though can show less side effects (Table 3). Higher value of index is the indication of multi-functionality of AMP i.e. it will interact with extreme range of proteins.

5.11 Cell penetrating ability

Cell penetration ability of multiple AMP sequences was determined by CellPPD tool (http://crdd.osdd.net/raghava/cellppd/multi_pep.php). **S**tructures and its mechanism of action regarding AMPs have vast array of diversity. AMPs should interact with bacterial cell surface which will induce perturbations in the plasma membrane. It will release cytoplasmic contents into environment by disrupting electrochemical gradients of membrane through structural changes, pores formation or membrane solubilisation. All these ten AMPs and their matching APD2 derived AMPs are non cell penetrating antimicrobial peptide. Support Vector Machine (SVM) score of these AMPs are viz. AP00350 (-0.7), AP01974 (-0.53), AP00183 (-0.3), AP02427 (-0.1), AP01975 (-0.09), AP00361 (0.01), AP00359 and AP00360 (0.02), AP00373 (0.16) and AP00147 (0.19). Barrel-stave, Toroidal pore, In-plane diffusion, Carpet, electroporation and Sinking raft [34], models were explained for mechanism of AMPs by using pore formation in target cell membrane. Negative value of SVM score indicates very poor cell penetration ability and positive values for good cell penetrating ability. The values when becomes more positive it will result into cell penetration ability of AMP. AP00373 and AP00147 have better cell penetration ability (Table 3).

5.12 Antigenicity, Allergic potential and Toxicity

Antimicrobial peptides should not be allergic, antigenic or toxic to the human, although it has broad spectrum antimicrobial activity. Antigenic peptide prediction tool (Immunomedicine Group) (http://imed.med.ucm.es/Tools/antigenic.pl) was used for determining presence of antigenic determinants (epitopes) within the AMPs. Antigenicity is predicted by using simple method, which is based on occurrence of amino acid

residues in experimentally determined epitopes [35]. Only AP01975 was free from epitopes, while in other eight AMPs single epitope was observed *viz.* AP00147, AP00350, AP00359, AP00360, AP00361, AP00373, AP01974 and AP02427. Two epitopes were observed within AP00183 AMP (Table 4). EVALLER™ web-tool (http://www.slv.se/en-gb/Group1/Food-Safety/e-Testing-of-protein-allergenicity/e-Test-allergenicity/) was used for determining allergic potential using e-Testing, which is based on amino acid sequence of AMP. All these AMPs are none allergic (Table 5). ToxinPred tool (http://crdd.osdd.net/raghava/toxinpred/protein.php) was used for predicting regions in AMP, which contributes in toxicity. All these AMPs are nontoxic (Table 5).

5.13 Intestinal half-life

HLP tool (http://www.imtech.res.in/raghava/hlp/pep_both.htm) was used to predict the half-life of AMP in the intestine like environment. Oral dose of drug was one of the best routes for drug delivery because of its low cost and minimum infection prone to inappropriate uses as well as that was readily accepted by the patients. Values of half-life of AMPs in the intestinal environment are given in seconds and prediction was in terms of stability as high, normal or low. As per time in seconds half-life shown by these AMPs are shown in Table 5. Physicochemical properties of AMPs viz. larger molecular weight, higher susceptibility to proteases and other enzymes, problems in renal as well hepatic clearance are the problems, hence maintaining its stability is difficult task [36].

5.14 Helix Wheel Diagrams

Helical Wheel Projections tool (www.rzlab.ucr.edu/scripts/wheel.cgi?sequence) of RZ LAB was used for helix wheel diagram prediction, represented the helix amphiphilicity as 2D 'helical wheel' diagram, which shows projections down to the helix axis and their relative orientation with amino acid residues. Hydrophobic movement was explained by Eisenberg, Weiss and Terwillig, which is a quantitative measurement of amphiphilicity perpendicular to the axis of secondary structure segments. Even distribution of hydrophilic and hydrophobic amino acids will give rise to less hydrophobic movement scale, while if arrangement of hydrophobic amino acid residues as well as hydrophilic amino acid residues are in the opposite pole on the sides of helix, hydrophobic movement scale increases. Angle of hydrophobic movement is the site where secondary structure comes in contact with cell membrane phospholipid. Hydrophobic movement is a determination of peptide's amphipathicity, which is an average of vectorial sum of all amino acid residues within ideal helix [31]. Higher permeabilization as well as hemolytic activity was obtained by peptides those have greater hydrophobic movement scale. Hydrophobic movement scales doesn't street forwardly correlates to all peptide secondary structures because of uneven distribution of hydrophobic as well as hydrophilic amino acid residues, hence ideal helices were not formed with membranes [37]. Binding of AMP requires hydrophobic surface that could penetrate in cell membrane cores made up from non-polar acyl (fatty acids) chains and hydrophilic surface should remain attached to polar head of phospholipid. Cell membrane binding and perturbation of peptide was considered as polar angle. AMPs having smaller polar angle has greater hydrophobic facets, which is directly proportional to better permibilization and faster pore forming ability, but these have shorter half-life compared with larger polar angle [31]. Probability of AMPs to act as trans-membrane peptides generally should possess lower hydrophobic moment values than 0.2. All of these AMPs are >1.21 to <6.27 hydrophobic movement scales, hence may be less penetration ability in the plasma membrane of target cell. Hydrophobic movement scales and hydrophobic movement angles of these ten AMPs are as per fig.3.

5.15 Secondary structure prediction

SAS tool (http://www.ebi.ac.uk/thornton-srv/databases/cgi-bin/sas/) of EMBL-EBI was used for prediction of secondary structures of AMPs. AMPs are grouped on the basis of its secondary structure as α-Helix, β-Sheet and Complex. AP00147, AP00183, AP00350, AP00373, AP01975 and AP02427 peptides belongs to α-helix group, while AP00359, AP00360, AP00361 and AP01974 possess β-sheet type of secondary structure (Fig.4). Initially α-Helix AMPs were identified and characterized. These are nonstructural peptides in aqueous environment, while adopt α-helix upon interaction with biological membranes, which allows insertion and cause permeabilization of the target cell membrane [38]. Most significant and studied examples of this class are the cecropins, magainins and melittins. A single β-hairpin structure displaying AMPs are kept under β-sheet group. These AMPs have simple secondary structures due to presence of single or double disulfide bridges. These structures have been already detected in aqueous environment; though will be further stabilized upon interaction with bacterial membrane. Tachyplesin and protegrin [39], are the representative examples of β-sheet AMPs. Complex AMPs has contained properties of α-helix and β-sheet amino acid residues' orientation. These AMPs show either α-helix or β-sheet forms, None of AMP belongs to complex group.

[4]. CONCLUSION

The inferences came out on the basis of bioinformatics analysis will be helpful for the drug designing, to study the diversity of AMPs, to study the physico-chemical properties and its effect on activity of AMPs and using silkworm for synthesis of AMPs. Clinical trials and Drug release in the market will take prolonged period, which can be reduced using such type of study.

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Compliance with Ethical standards

Ethical approval:

This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of interest: All authors have no conflict of interest for publication.

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FIGURES AND TABLES

Table 1: Matching of AMPs with AMPer Database antimicrobial peptides

Group	Peptide family	Lowest HMM E-value	Blast E-value	% Identity
AP00147 HMM matches to mature peptide				
22	Moricin, Virescein	1.8e-30	7e-21	100
40	Lycotoxin, Dermaseptin	0.0003	-	-
73	Dermaseptin, Dermatoxin, Magainins	0.0031	-	-
AP00183 and AP02427 HMM matches to mature peptide				
51	Cecropin	2.8e-25	2e-17	94.29
104	Cecropin-A, Hyphancin, Moricin	1.6e-23	3e-15	77.14
111	Maximins, Ponericin	0.0031	-	-
139	Bactericidin, Cecropin, Hyphancin	1e-19	4e-17	100
142	Cecropin, Sarcotoxin	0.00021	0.002	47.37
AP00183 and AP02427 HMM matches to Propeptide				
1	Neutrophil defensin 3,4	0.0065	-	-
AP00350 HMM matches to mature peptide				
51	Cecropin	5e-13	2e-08	59.38

104	Cecropin-A, Hyphancin, Moricin	3.6e-26	1e-18	100
139	Bactericidin, Cecropin, Hyphancin	9.2e-17	9e-11	65.71
142	Cecropin, Sarcotoxin	0.0002	0.008	52.63
AP00359 HMM matches to mature peptide				
26	Sarcotoxin	3.7e-05	-	-
72	Lebocin	4.8e-27	7e-17	100
AP00360 HMM matches to mature peptide				
26	Sarcotoxin	0.00035	-	-
72	Lebocin	9.2e-28	6e-17	100
AP00361 HMM matches to mature peptide				
26	Sarcotoxin	0.00044	-	-
72	Lebocin	3.8e-28	2e-17	100
AP00373 HMM matches to mature peptide				
22	Moricin, Virescein	3.7e-32	9e-22	100
40	Lycotoxin, Dermaseptin	0.0003	-	-
73	Dermaseptin, Dermatoxin, Magainins	0.0031	-	-
AP01974 and AP01975 HMM matches to mature peptide				
-	-	-	-	-

Table 2: Analysis of AMPs using ProtParam tool

APD ID	**AP0-**									
	0147	**0183**	**0350**	**0359**	**0360**	**0361**	**0373**	**1974**	**1975**	**2427**
Total amino acids										
	42	35	37	32	32	32	42	21	18	37
Amino acid composition (%)										
Ala(A)	16.7	11.4	24.3	0.0	0.0	0.0	14.3	9.5	11.1	10.8
Arg(R)	4.8	8.6	10.8	9.4	9.4	9.4	4.8	4.8	5.6	8.1
Asn(N)	2.4	2.9	2.7	6.2	6.2	9.4	7.1	0.0	5.6	2.7
Asp(D)	2.4	2.9	2.7	6.2	6.2	6.2	2.4	4.8	5.6	2.7
Cys(C)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Gln(Q)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	9.5	11.1	0.0
Glu(E)	0.0	5.7	0.0	0.0	0.0	3.1	0.0	0.0	0.0	5.4
Gly(G)	7.1	11.4	5.4	6.2	6.2	3.1	7.1	9.5	0.0	13.5
His(H)	0.0	0.0	2.7	0.0	0.0	0.0	2.4	4.8	0.0	0.0
Ile(I)	11.9	17.1	0.0	6.2	6.2	6.2	11.9	4.8	5.6	16.2
Leu(L)	4.8	2.9	8.1	9.4	12.5	12.5	4.8	0.0	0.0	2.7
Lys(L)	21.4	17.1	0.0	6.2	6.2	6.2	21.4	0.0	11.1	18.9
Met(M)	0.0	2.9	2.7	3.1	3.1	3.1	0.0	0.0	5.6	2.7
Phe(F)	4.8	2.9	0.0	6.2	6.2	6.2	4.8	0.0	16.7	2.7
Pro(P)	4.8	2.9	2.7	25.0	21.9	21.9	4.8	0.0	5.6	2.7
Ser(S)	2.4	2.9	5.4	0.0	0.0	0.0	2.4	14.3	5.6	2.7
Thr(T)	4.8	0.0	8.1	3.1	3.1	3.1	4.8	9.5	5.6	0.0
Trp(W)	0.0	2.9	2.7	0.0	0.0	3.1	0.0	4.8	0.0	2.7
Tyr(Y)	0.0	0.0	0.0	9.4	9.4	6.2	0.0	19.0	0.0	0.0
Val(V)	7.1	5.7	13.5	3.1	3.1	0.0	7.1	4.8	5.6	5.4
Asp + Glu	1	3	3	2	2	3	1	1	1	3
Arg + Lys	11	9	5	5	5	5	11	1	3	10
pI[a]	11.36	10.64	10.67	9.82	9.82	9.52	11.36	6.74	9.99	10.71

Instability index	8.32	67.27	42.39	37.09	37.09	35.12	8.32	38.77	62.19	61.88
Aliphatic index	102.38	106.0	95.14	70.00	82.19	73.12	100.00	41.90	48.89	100.27
GRAVY[b]	-0.09	-0.13	0.232	-0.67	-0.50	-0.82	-0.210	-0.79	-0.38	-0.243
In vivo half-life (Hours)										
Reticulocyte	4.4	1	>20	1.1	1.1	1.1	4.4	2.8	1.3	1
Yeast	>20	2 min	>20	3 min	3 min	3 min	>20	10 min	3 min	2 min
E. coli	>10	2 min	?	>10	>10	>10	>10	2 min	3 min	2 min

Note: [a]: Isoelectric point and [b]: Grand average of hydropathicity

Table 3: Net charge, hydrophobicity, Boman index and Cell penetrating ability of AMPs

APD ID	Charge	hydrophobicity	Boman index (kcal/mol)	Cell penetrating ability	
				SVM Score	Prediction
AP00147	10	45%	1.16	0.19	Non-CPP
AP00183	6	45%	1.4	-0.30	Non-CPP
AP00350	2	51%	1.47	-0.70	Non-CPP
AP00359	3	28%	1.58	0.02	Non-CPP
AP00360	3	31%	1.43	0.02	Non-CPP
AP00361	2	31%	1.93	0.01	Non-CPP
AP00373	10	42%	1.31	0.16	Non-CPP
AP01974	0.50	23%	1.83	-0.53	Non-CPP
AP01975	2	44%	1.92	-0.09	Non-CPP
AP02427	7	43%	1.45	-0.10	Non-CPP

Table 4: Antigenicity (epitopes) of AMPs

APD ID	Start position	Sequence	End position
AP00147	4	PIKAIKTVGK	13
AP00183	16	RDGIVKA	22
	24	PAIEVLGS	31
AP00350	8	IERAVARTRDAVISAGPAVRTVAAAT	33
AP00359	4	FLYPRGKLPVPTPPPFNPKPIY	25
AP00360	4	FLYPRGKLPVPTLPPFNPKPIY	25
AP00361	9	EKLPLPTLPPFNPKPIY	25
AP00373	4	PIKAIKTVGK	13
AP01974	4	STHAVIYAQGY	14
AP01975	0	**none antigenic**	0
AP02427	16	RDGIVKAGPAIEVLGS	31

Table 5: Allergenic potential, toxicity and intestinal half-life of AMPs

APD ID	Allergic potential		Toxicity	Half-life in the intestine	
	% possibility	Prediction		in seconds	Stability
AP00183	0.4	No allergic	Nontoxic	0.147	Normal
AP00350	1.2	No allergic	Nontoxic	0.0001	Low
AP00359	0.8	No allergic	Nontoxic	0.343	Normal
AP00360	2.4	No allergic	Nontoxic	0.127	Normal
AP00361	1.6	No allergic	Nontoxic	0.096	Low
AP00373	8.0	No allergic	Nontoxic	0.373	Normal
AP01974	0.4	No allergic	Nontoxic	0.332	Normal
AP01975	0.4	No allergic	Nontoxic	0.0001	Low
AP02427	1.2	No allergic	Nontoxic	0.147	Normal

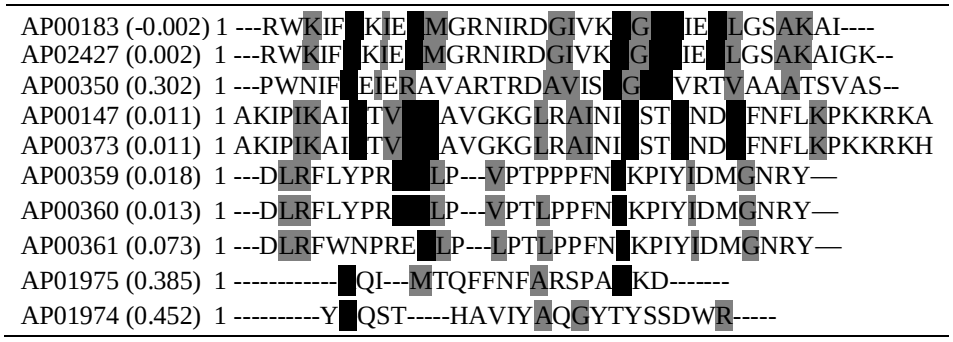
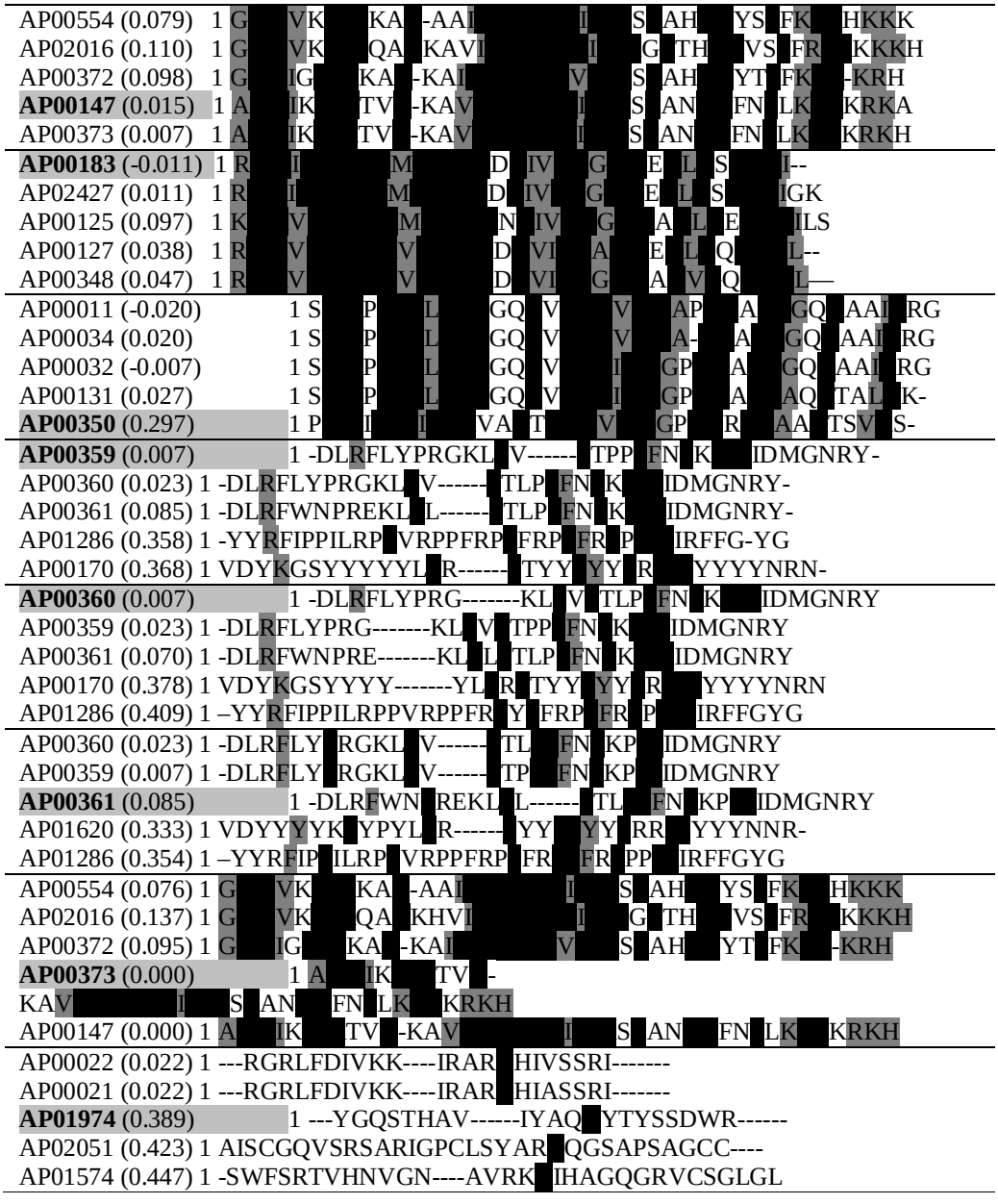


Figure 1: Conserved amino acid pattern



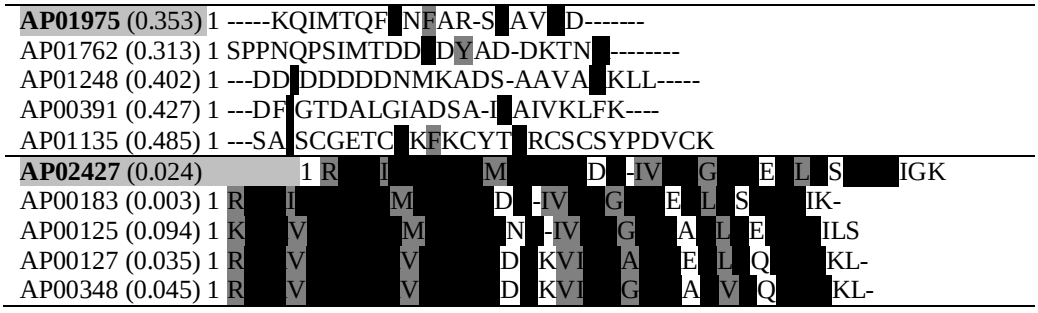


Figure 2: Sequence Homology of AMPs

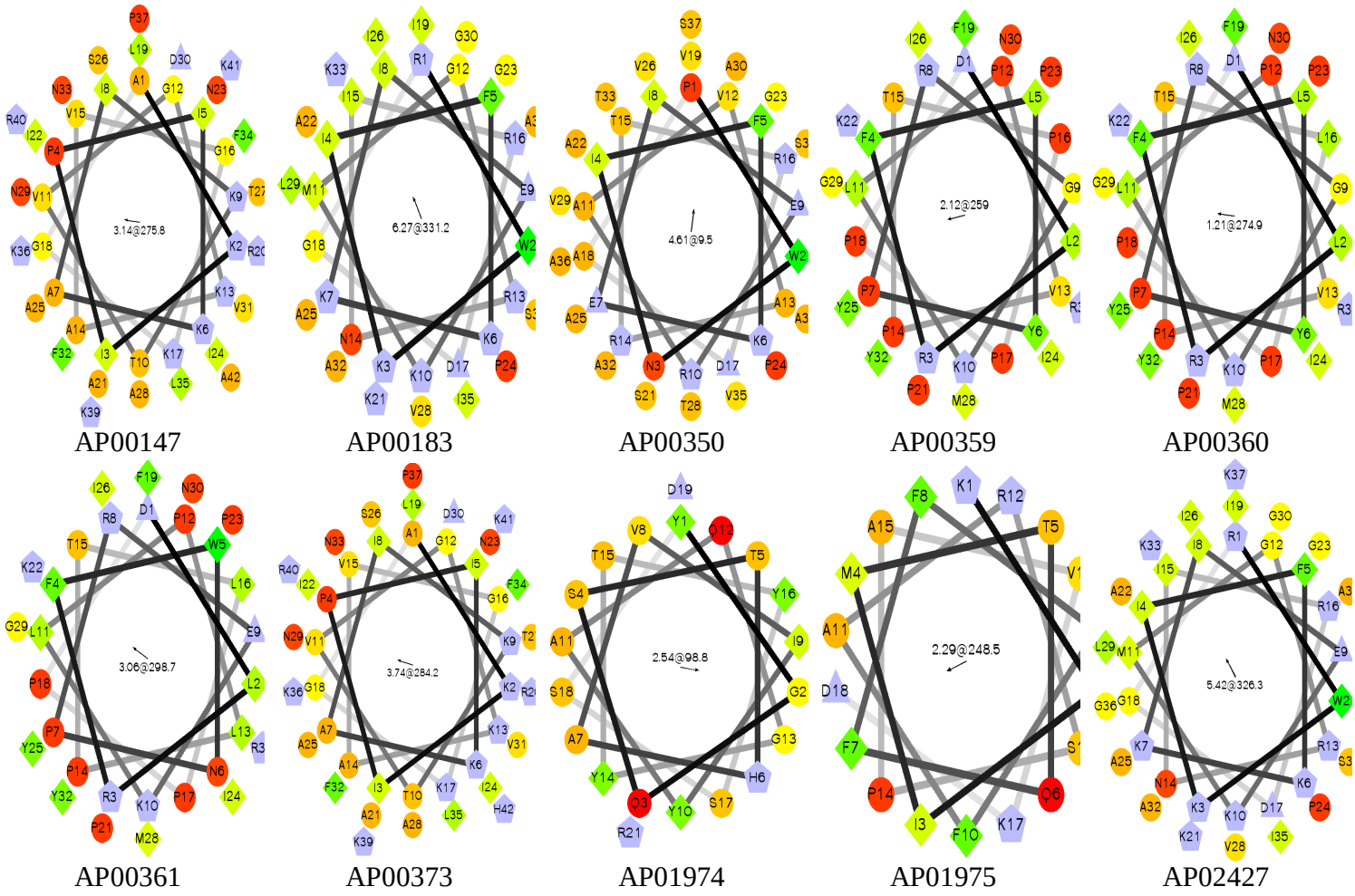


Figure 3: Helix Wheel diagrams of AMPs :Shapes and colors represents nature of amino acid residues as circles (Hydrophilic), diamonds (Hydrophobic), triangles (Potentially negative), pentagon (Potentially positive), dark green (most hydrophobic), faint green (decreased hydrophobicity), yellow (nil hydrophobicity), pure red (uncharged hydrophilic), faint red (decreased hydrophilicity) and light blue (potentially charged). At the centre of each wheel diagram hydrophobic movement scale (HM scale) and hydrophobic movement angle (HM angle) are separated by sign @.

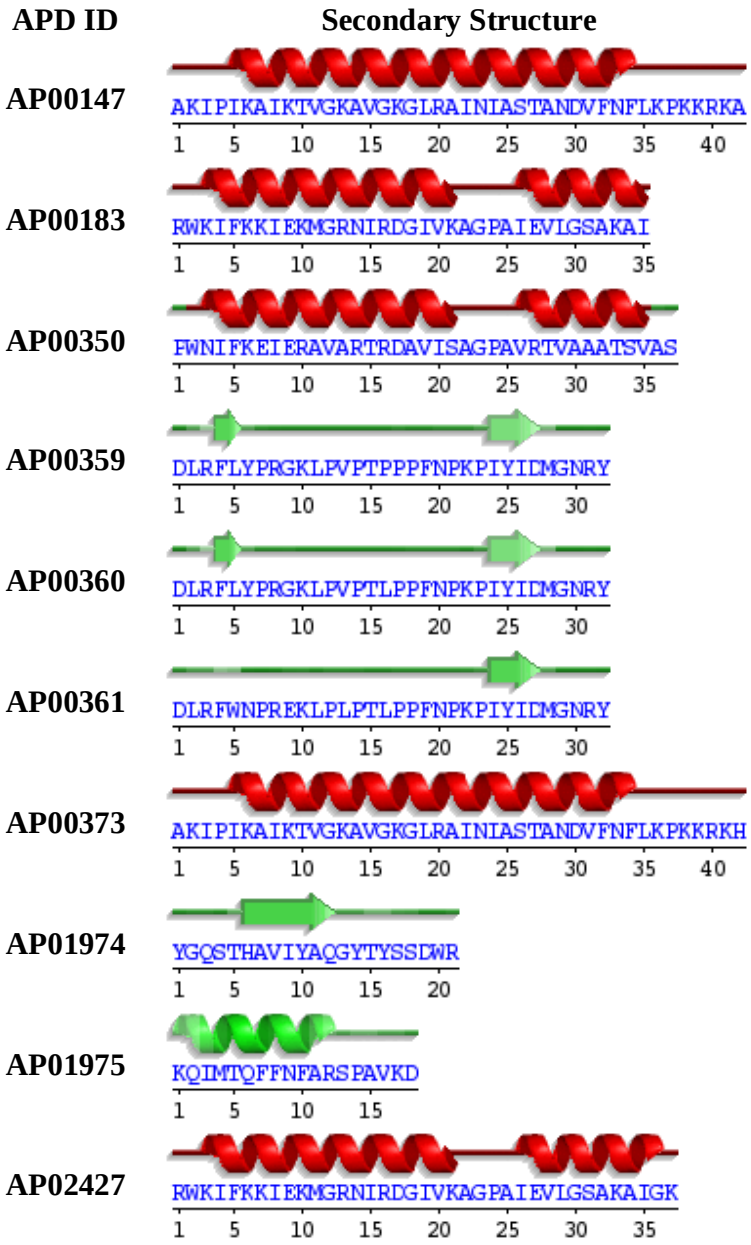


Figure 4: Secondary structures of AMPs: Red color represents homologous regions with proteins of known 3D structure in the Protein Data Bank (PDB).