

AVPpred: collection and prediction of highly effective antiviral peptides

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ABSTRACT

In the battle against viruses, antiviral peptides (AVPs) had demonstrated the immense potential. Presently, more than 15 peptide-based drugs are in various stages of clinical trials. Emerging and re-emerging viruses further emphasize the efforts to accelerate antiviral drug discovery efforts. Despite, huge importance of the field, no dedicated AVP resource is available. In the present study, we have collected 1245 peptides which were experimentally checked for antiviral activity targeting important human viruses like influenza, HIV, HCV and SARS, etc. After removing redundant peptides, 1056 peptides were divided into 951 training and 105 validation data sets. We have exploited various peptides sequence features, i.e. motifs and alignment followed by amino acid composition and physicochemical properties during 5-fold cross validation using Support Vector Machine. Physicochemical properties-based model achieved maximum 85% accuracy and 0.70 Matthew's Correlation Coefficient (MCC). Performance of this model on the experimental validation data set showed 86% accuracy and 0.71 MCC which is far better than the general antimicrobial peptides prediction methods. Therefore, AVPpred—the first web server for predicting the highly effective AVPs would certainly be helpful to researchers working on peptide-based antiviral development. The web server is freely available at <http://crdd.osdd.net/servers/avppred>.

INTRODUCTION

There has been a considerable focus per se on the antiviral research from the past decade due to the limited availability of therapeutic molecules for many viral infections (1). Apart from existing drugs and vaccines, there is a need to explore new antiviral candidates to control pathogenic re-emerging and resistant viruses (2). Antiviral peptides

(AVPs) are a potential alternative strategy in this context (3).

AVP were experimentally proven in blocking virus attachment or entry into host cells or inhibiting viral replication. In other words, these may interfere with the key steps of pathogenic human viruses (4). These inhibitory peptides could then be used as starting point for the design of more active molecules targeting the generic steps involved in virus attachment, fusion and replication, etc. These peptides may sometimes be preferable because of their relatively low molecular weight, lesser toxicity, rapid elimination from the host, lesser side effects and also cost effective synthesis nowadays (5).

Many workers have reported highly efficient peptides against human viruses, e.g. influenza (6–8), HIV (9,10), WNV (11), HCV (12), Rabies (4), HSV (13) and RSV (14), etc. Synthetic analogues of several naturally occurring antimicrobial peptides have been made in an attempt to identify important structural features contributing to the antiviral activity (15). Currently, 15 antimicrobial peptides have entered clinical trials (16). The importance of antimicrobial peptides has been further reviewed (17–20).

Despite immense potential of this field, there is no virus specific AVP prediction algorithm available. Although some general antimicrobial peptide prediction servers exist like CAMP (21), APD2 (22), AntiBP2 (23) and Wang *et al.* (24). At the same time, presently there is no dedicated database pertaining to AVPs. As far as AVP sequences are concerned, collectively less than 200 AVPs are provided in the functional antimicrobial peptide database APD2 (22) and CAMP (21). In the present study, we have developed the first AVP prediction method based on the collected peptides which were experimentally proven for antiviral activity.

MATERIALS AND METHODS

Algorithm development

Data collection

We have screened several hundred research articles and patents indexed in Pubmed and Patent Lens, respectively, to retrieve AVP sequences. From more than 80 relevant

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articles; we have extracted 1245 peptide sequences with a reported antiviral activity against human viruses like HIV, HCV, SARS and Influenza, etc. About 150 AVP of our collection were also present in the existing antimicrobial databases (21,22). Nearly 91% of the collected peptides were from natural source and remaining have synthetic source. Identical peptides were removed to finally have 604 highly effective and 452 least or non-effective AVPs which were used in the training $T^{544p+407n}$ (544 positive and 407 negative) and validation $V^{60p+45n}$ (60 positive and 45 negative) data sets, respectively. Simultaneously, we have made another training $T^{544p+544n*}$ and validation $V^{60p+60n*}$ data sets where non-experimental negative peptides [as employed in earlier antimicrobial peptide prediction method (23)] were used in place of experimentally verified negative data set.

Peptide features

General antimicrobial peptide prediction methods exploited several peptide sequence features in the past. We have also utilized the most important features like amino acid composition and physicochemical properties for SVM model development and also used sequence alignment technique. We have included another important sequence motif feature which was not exploited earlier in the AMP predictions.

Motif search

Motif-based approach has been used in past for protein and peptide identification (25,26). However this approach was not exploited for finding antimicrobial peptides so we searched for the conserved motifs in the AVP using MEME/MAST (27,28), where MEME is used to discover motifs and MAST is used to search these motifs in peptides. Correct prediction of MEME/MAST depends on *E*-values. If at given *E*-value MAST searches any motif then this peptide is predicted as AVP and if not found then it is predicted to be non-AVP. Firstly we extracted 20 motifs (as provided in the Supplementary Table S1) from AVP using MEME and searched these motifs in antiviral and non-AVPs using MAST at various *E*-values and best results were found at *E*-value 10.

Sequence alignment

The sequence segments with high identity are inclined to share the structure and function. This method has been widely used in the past for proteins and peptide prediction, like in signal peptide prediction using BLASTP (29). Similarly Wang *et al.* (24) used BLASTP for prediction of antimicrobial peptide. We have also implemented BLASTP algorithm for prediction of AVPs. Briefly we created two databases; antiviral database having 544 and Non-Antiviral database having 407 peptides. Both databases were then formatted into BLAST readable format using 'formatdb'. Optimized parameters were used as reported earlier (24). Each query sequence was matched against antiviral and non-AVP databases using BLASTP program. If the most significant hit is found in the antiviral database then peptide is predicted to be AVP else non-AVP if hit is found in the non-antiviral database.

Amino acid composition

Amino acid composition is the fraction of each amino acid in a peptide. The fraction of all 20 natural amino acids was calculated using the following equation:

Fraction of amino acid X = Total no. of X/peptide length.

Physicochemical properties

Antimicrobial peptide properties cannot be defined by amino acid sequence pattern alone, various combination of structural and physicochemical features like secondary structure, overall charge, size, residue composition, hydrophobicity and amphiphilic character are responsible for overall activity of AMP (30). Torrent *et al.* (31) has used physicochemical properties for the analysis and prediction of antimicrobial peptides and have shown good results. We tried all 544 physicochemical properties available in AAINDEX database (32) individually, to look into the importance of each property in antiviral activity of the peptides. Model of 544 physicochemical properties was not implemented due to its very large (10 880) vector size. Finally selected 25 best performing physicochemical properties were used in developing AVPphysico model as provided in the Supplementary Table S2.

Implementation

The Support Vector Machine (SVM) approach has been used extensively in the areas of pattern recognition and classification (26). SVM is a learning algorithm which, upon training with a set of positively and negatively labelled samples, produces a classifier that can then be used to identify the correct label for unlabelled samples. The best performance was obtained using the RBF kernel parameters. For the present study, we used the freely downloadable SVM^{light} package.

Validation

In the present study, 5-fold cross validation technique has been adopted to evaluate the performance of the various SVM modules constructed. In this technique, the data set was partitioned randomly into five equally sized sets. The training and testing was carried out five times, each time using one distinct set for testing and remaining four sets for training. The following equations were often used in literatures to reflect the prediction quality:

$$\text{Sensitivity } (S_n) = [TP/(TP+FN)] \times 100$$

$$\text{Specificity } (S_p) = [TN/(TN+FP)] \times 100$$

$$\text{Accuracy } (A_c) = [TP+TN/(TP+FP+TN+FN)] \times 100$$

$$MCC = \frac{TP \times TN - FP \times FN}{\sqrt{(TP+FP)(TP+FN)(TN+FP)(TN+FN)}}$$

where S_n reflects the sensitivity, S_p the specificity, A_c the accuracy and MCC, the Mathew's correlation coefficient; while TP represents the true positive, TN, the true negative; FP, the false positive and FN, the false negative. S_n , S_p and A_c stand for the success rates of prediction on positive, negative and overall data sets, respectively. MCC is used to evaluate the performance of the predictor

when the positive and negative samples in the data set are out-of-balance. Its value ranges from -1 to 1 and a larger MCC means a better prediction.

RESULTS

Performance evaluation during 5-fold cross validation

During 5-fold cross validation, all four AVP prediction models were evaluated, namely AVPmotif based on 20 AVP motifs; AVPalign based on sequence alignment; AVPcompo based on amino acid composition and AVPphysico based on 25 physico-chemical properties. All models were made on data set $T^{544p+407n}$. Similarly four new models (suffixed by #) as above were developed on another data set $T^{544p+544n*}$ having non-experimental peptides. Performances of all these models are shown in the Table 1.

Performances of all the models were very good. AVPmotif model based on antiviral motifs also performed well; however, we achieved maximal 85% accuracy and 0.70 correlation on $T^{544p+407n}$ experimental data set by AVPphysico model using sequence physicochemical features. AVPalign model also performed equally well. On the other $T^{544p+544n*}$ data set, amino acid composition feature-based model AVPcompo# showed best performance with 90% accuracy and 0.81 correlation. When comparing the results on both the data sets, it is observed that all four models developed on $T^{544p+544n*}$ data set performed slightly better than the models developed using $T^{544p+407n}$ complete experimental data set during 5-fold cross validation. This could be attributed to the fact that $T^{544p+544n*}$ data set contain non-experimental peptides in the negative data set. These negative peptides were generated from the non-secretary proteins which have least chance of being positive and enhancing the model accuracy. We have also checked the performance of a hybrid model by combination of all four methods during 5-fold cross validation and on independent validation data set. However, performance of the hybrid model is almost similar to that of best individual method.

Performance evaluation on independent data set

Since 5-fold cross validation evaluation is not considered sufficient, therefore, we have also evaluated the

Table 1. Performance of AVPpred models during 5-fold cross validation

Data set	Model	Sensitivity	Specificity	Accuracy	MCC
$T^{544p+407n}$	AVPmotif	72.3	82.2	76.8	0.54
	AVPalign	88.3	81.0	85.0	0.70
	AVPcompo	80.7	87.0	83.4	0.67
	AVPphysico	82.2	88.2	85.0	0.70
$T^{544p+544n*}$	AVPmotif#	72.3	88.4	80.4	0.62
	AVPalign#	86.0	92.7	89.3	0.79
	AVPcompo#	84.2	96.1	90.2	0.81
	AVPphysico#	89.7	90.3	90.0	0.80

Training data set $T^{544p+407n}$ is consisting of experimental AVP, while training data set $T^{544p+544n*}$ was containing non-experimental peptides. #AVP models developed using $T^{544p+544n*}$ data set.

performance of our models on the validation/independent data set. This data was not included anywhere in the training or testing. There were two validation data sets: (i) $V^{60p+45n}$ having complete experimental verified 105 peptides and (ii) $V^{60p+60n*}$ data sets with non-experimental negative peptides (see material and method). Each of these independent data set was evaluated using four models developed using $T^{544p+407n}$ and $T^{544p+544n*}$ data sets, respectively. Detailed results are shown in Table 2.

On the $V^{60p+45n}$ independent data set both AVPcompo and AVPphysico method achieved maximum accuracy of 86% and 0.71 MCC while on the same data set AVPphysico# model showed 74% accuracy and 0.48 MCC. Similarly on $V^{60p+60n*}$ independent data set AVPcompo model performed best with 86% accuracy and 0.72 MCC while AVPphysico# also outperformed other models with 93% accuracy and 0.85 MCC.

On both the independent data set, performance of the AVP models developed on $T^{544p+407n}$ data set were quite good and similar. At the same time, results also revealed that all models except AVPmotif which were developed on the experimental $T^{544p+407n}$ data set outperformed the models developed on the $T^{544p+544n*}$ data set having non-experimental peptides on complete experimental data. This suggests that it may be better to include experimental verified negative peptides data also for the antimicrobial peptides prediction.

Comparison with existing antimicrobial peptide prediction algorithms

It is not appropriate to compare the AVPpred with any other method since none such method available. Still in the context of antimicrobial peptide prediction algorithm we made the comparison with latest AMP prediction methods of Wang *et al.* (24) and Thomas *et al.* (21). Results are shown in the Table 3.

Results showed that on the $V^{60p+45n}$ complete experimental validation data set, best models of Wang *et al.* (24)

Table 2. Performance of AVPpred models on validation/independent data sets $V^{60p+45n}$ and $V^{60p+60n*}$

Data set	Model	Sensitivity	Specificity	Accuracy	MCC
$V^{60p+45n}$	AVPmotif	70.0	77.8	73.3	0.47
	AVPalign	81.7	82.2	81.9	0.63
	AVPcompo	83.3	88.9	85.7	0.72
	AVPphysico	88.3	82.2	85.7	0.71
	AVPmotif#	70.0	81.7	75.8	0.52
	AVPalign#	83.3	48.9	68.6	0.35
	AVPcompo#	83.3	62.2	74.3	0.47
	AVPphysico#	93.3	48.9	74.3	0.48
	$V^{60p+60n*}$	AVPmotif	70.0	81.7	75.8
AVPalign		81.7	78.3	80.0	0.60
AVPcompo		83.3	88.3	85.8	0.72
AVPphysico		88.3	65.0	76.7	0.55
AVPmotif#		70.0	83.4	76.7	0.58
AVPalign#		83.3	95.0	89.2	0.79
AVPcompo#		83.3	98.3	90.8	0.83
AVPphysico#		93.3	91.7	92.5	0.85

$V^{60p+60n*}$ was containing non-experimental peptides.

#AVP models developed using $T^{544p+544n*}$ data set.

Table 3. Comparison of AVPpred models with recent antimicrobial peptide prediction methods on independent data sets $V^{60p+45n}$, $V^{60p+60n*}$ and $T^{604p+452n}$

Data set	Model	Sensitivity	Specificity	Accuracy	MCC
$V^{60p+45n}$	Wang <i>et al.</i> (24)	61.7	80.0	69.5	0.42
	Thomas <i>et al.</i> (21)	40.0	77.3	55.8	0.18
$V^{60p+60n*}$	Wang <i>et al.</i> (24)	61.7	90.0	75.8	0.54
	Thomas <i>et al.</i> (21)	40.0	86.7	63.3	0.30
$T^{604p+452n}$	Wang <i>et al.</i> (24)	56.1	75.4	64.4	0.32
	Thomas <i>et al.</i> (21)	37.0	76.8	54.1	0.15

$T^{604p+452n}$ data set is consists of both experimental training and validation data sets.

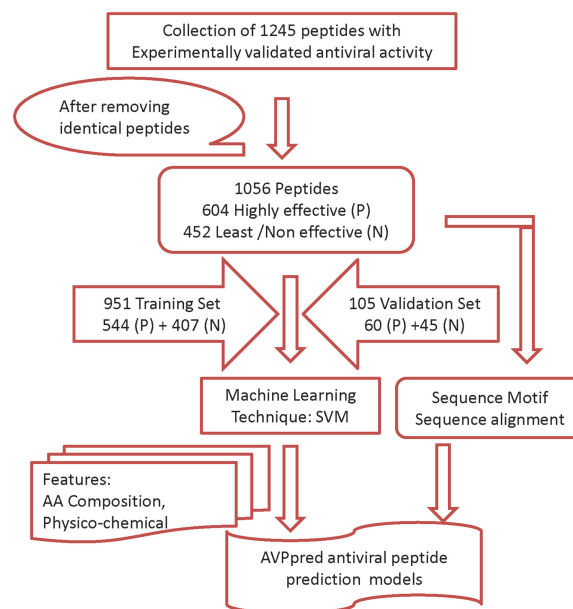
method could achieve only 70% accuracy and 0.42 MCC despite the fact that 15 of these positive peptides were also included in the Wang *et al.* (24) training model while model of Thomas *et al.* (21) performed rather poorly with 56% accuracy and 0.18 MCC. On the other side, our both AVPcompo and AVPphysico models based on complete experimental data performed far better with accuracy of 86% and 0.71 MCC on the same independent data set. Even overall performance of general AMP prediction method on the whole $T^{604p+452n}$ experimental data is not good. Similarly on the $V^{60p+60n*}$ independent data set, our AVPphysico[#] model outperformed the Wang *et al.* (24) model with 93% accuracy and 0.85 MCC. This is due to the fact that our method is specific for the AVPs than the existing general antimicrobial peptide prediction methods are mostly suitable for bacteria. It further suggests that AVP may have some unique features which our AVPpred algorithm best able to incorporate and thus outperformed other AMP prediction in case of AVPs.

Webserver

AVPpred web server is freely accessible via the URL <http://crdd.osdd.net/servers/avppred>. On the 'Home' page a general description of the server is provided. Documentation regarding usage and algorithm are available under 'Help' and 'Algorithm' pages. A flowchart depicting the workflow of AVPpred web server is shown in Figure 1. Data sets used in model development are also displayed in separate pages. A general outline of the server is displayed in Figure 2.

Input

To use this prediction model, an online web server available via the URL <http://crdd.osdd.net/servers/avppred> has been provided. On the submit page user may paste single or multiple peptide sequence(s) in FASTA format in the provided text-box or upload a FASTA file from the system. User can remove the entries in the text-box by using the clear button. An 'Example link' has been provided to help the users. If user has a protein sequence, then, an option for making peptide fragments of desired length and overlapping residues is provided. User can paste these peptide fragments in the 'Predict AVP' form.

**Figure 1.** AVPpred workflow. Flowchart of the AVP model development.

Output

Results of the prediction are shown in a tabular as well as graphical layout. In first three columns of the result Table; Sequence No, Peptide Sequence Name and Length are given. In fourth column BLAST option is provided to search similar peptide sequences in CAMP and AMP antimicrobial peptide databases and also in Uniprot. By clicking the properties column, some relevant properties of the peptide like charge, polarity, amino acid composition, hydrophobicity, preference for alpha/beta strand, etc. are displayed. In next columns prediction of Models 1–4 corresponding to motif search, sequence alignment, amino acid composition and physicochemical properties are shown. Eighth and ninth columns display the numerical value of SVM score. This is displayed in colour code as red (high), yellow (medium) and green (low). The graph displays the specificity of the peptides corresponding to the 'composition' and 'physicochemical' models. Also users can download the prediction results as a tab delimited text file.

Tools

Users may BLAST their peptide for similarity against APD2, CAMP and Uniprot. The result shows distribution of hits, their score, *E*-value and alignment with sequences with significant similarity will be displayed. Also user can predict AVPs from other databases (CAMP and APD2). Further users can check some relevant physicochemical properties like charge, polarity, amino acid composition, hydrophobicity, preference for alpha/beta strand, for their peptides. Also the collection of positive and negative data sets has been provided on the server along with their sequence, length, target virus and references. In the figures below is shown the development of prediction models (Figure 1) and general outline of the web server (Figure 2).

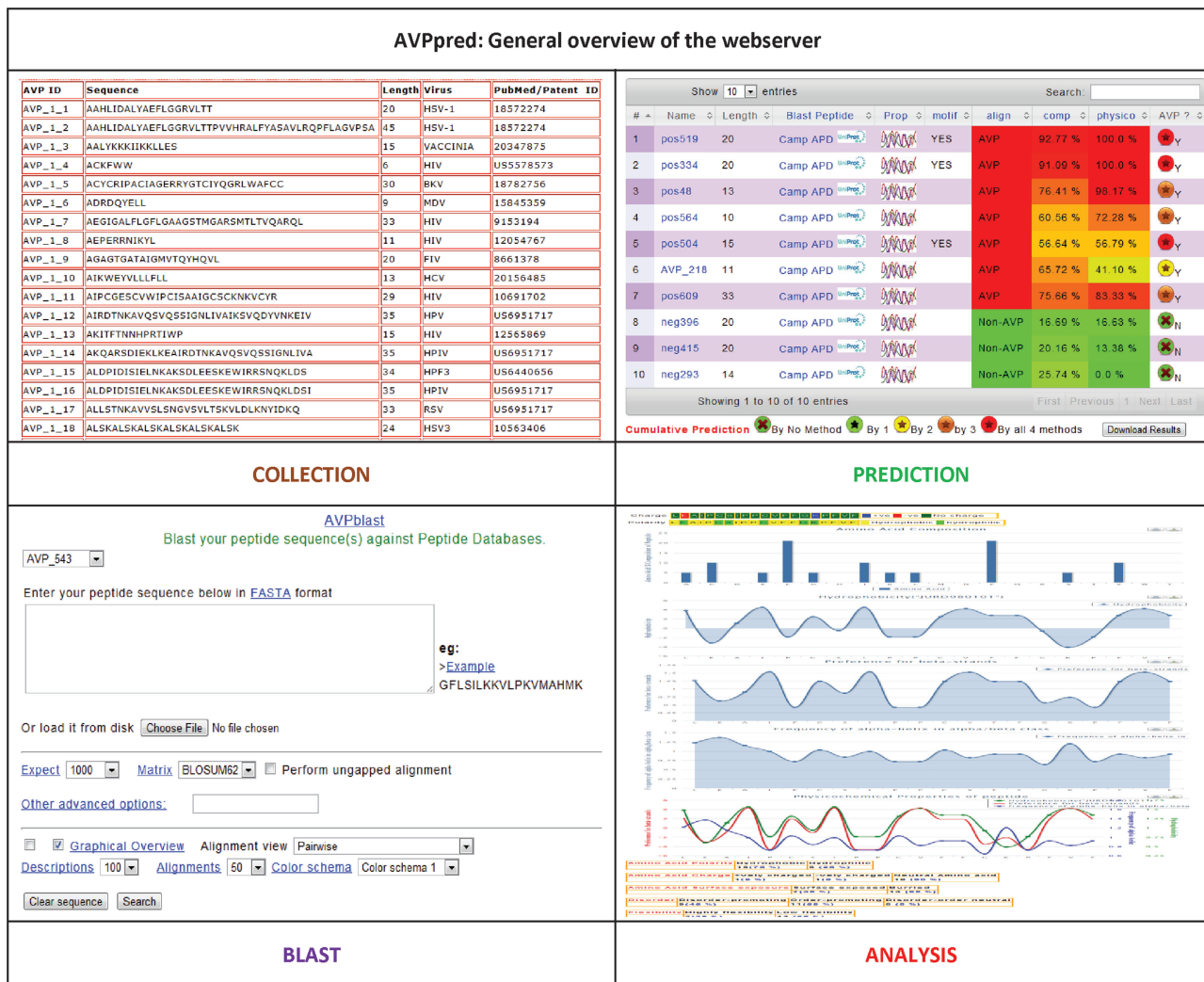


Figure 2. Web server overview. Outline of AVPpred web server and functionality.

Server implementation

The server AVPpred is implemented on Red Hat Linux and Apache (2.2.17) in back-end and front-end of web interface is implemented with PHP (5.2.14).

CONCLUSION

AVPpred is the first algorithm for prediction of highly effective AVPs based on experimentally validated positive and negative data sets. Four prediction models; the composition-based, physico-chemical properties and sequence alignment-based were implemented in the web server to make comprehensive predictions. This web server would be helpful for researchers working for the development of peptide-based antiviral therapeutics.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online: Supplementary Tables 1 and 2.

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