4th International Electronic Conference on Medicinal Chemistry
1-30 November 2018
chaired by Dr. Jean Jacques Vanden Eynde
sponsored by pharmaceuticals

DBAASP Special prediction as a tool for the prediction of antimicrobial potency against particular target species

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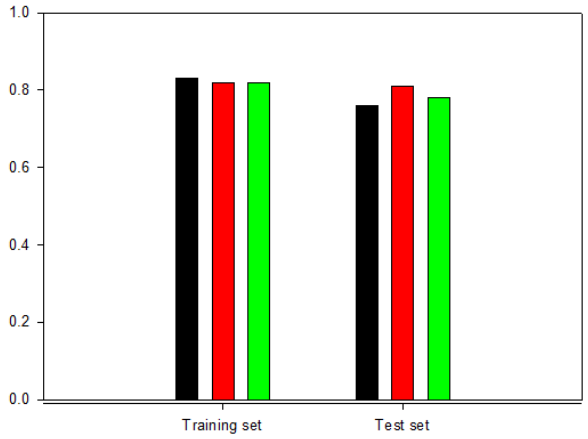
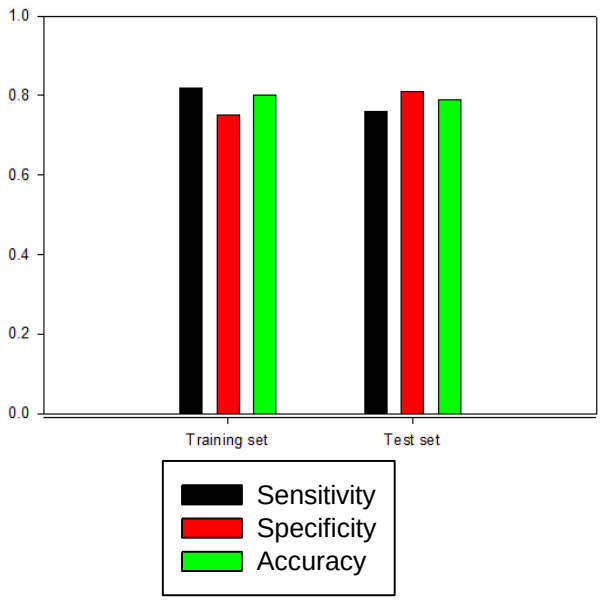
Graphical Abstract

Performances of Predictive models for

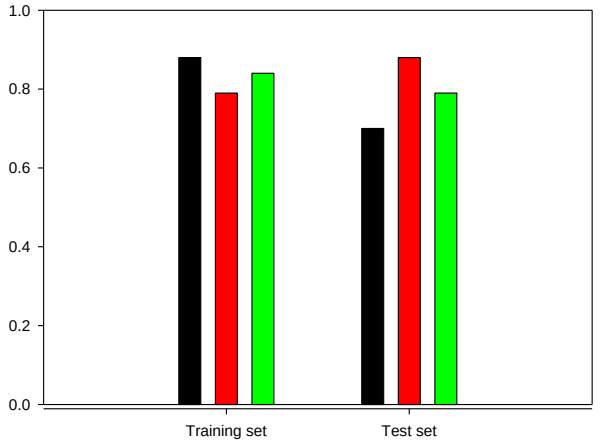
Staphylococcus aureus 25923



Bacillus subtilis



Human Erythrocytes



Abstract

Antimicrobial peptides (AMPs) have been identified as a potentially new class of antibiotics. There are a lot of computational methods of AMP prediction. Although most of them can predict antimicrobial potency against any microbe (microbe is not identified) with rather high accuracy, prediction quality of these tools against particular bacterial strains is low [1,2]. Special prediction is a tool for the prediction of antimicrobial potency of peptides against particular target species with high accuracy. This tool is included into the Database of Antimicrobial Activity and Structure of Peptides (DBAASP, https://dbaasp.org [3]). In this presentation we describe this tool and predictive models for some Gram positive bacterial strains (Staphylococcus aureus ATCC 25923 and Bacillus subtilis) and a model for the prediction of hemolytic activity. Predictive model for Gram negative Escherichia coli ATCC 25922 was presented earlier [2,4]. Special prediction tool can be used for the design of peptides being active against particular strain. To demonstrate the capability of the tool, peptides predicted as active against E-coli ATCC 25922 and Staphylococcus aureus ATCC 25923 have been synthesized, and tested *in vitro*. The results have shown the justification of using special prediction tool for the design of new AMPs

Keywords: Antimicrobial peptides; AMP prediction; Design of AMPs

Introduction

- The problem of bacterial resistance to antibiotics is one of the important tasks in microbiology.
- Antimicrobial peptides in most cases act on the membrane of bacteria, which complicates the development of resistance of microbes to them.
- Therefore, AMPs are good candidates for new antibiotics

Introduction

- Currently, antimicrobial peptides are being actively studied. The database DBAASP [3] consists of more than 11 500 AMPs and their number is constantly increasing.
- Nevertheless, antimicrobial peptides are rarely used in clinical practice.
- There are several reasons that prevent the active use of AMP as antibiotics:
    1. They can be available for the action of proteases for a short time and the peptides do not show antimicrobial activity.
    2. Many AMPs have hemolytic or cytotoxic activity.
    3. Their high cost price.

Introduction

- Despite this, the design and synthesis of new peptides actively continues (more than 75% of peptides in DBAASP are synthetic.) and some of them are in clinical trial.
- For task-oriented design of new AMPs, tools for prediction of antimicrobial activities of peptides are needed.
- At the moment many AMP prediction tools are available .
- Most of these tools can only predict if a peptide has any antimicrobial activity, but cannot predict antimicrobial potency against particular strains [1,2]

Introduction

- So one of the main problems in the design of new peptides is the lack of effective predictive models capable of showing high performance when designing new amino acid sequences with a high therapeutic effect against specific strains of bacteria.

- Special prediction (SP) is developed as a tool for the prediction of antimicrobial potency of peptides against particular target species and therefore can be considered as an attempt to resolve the above-mentioned problems

Results and discussion

- Predictive models of SP were based on clustering of peptides into groups of peptides (clusters) according to their physical-chemical properties.
- The following 9 characteristics are used to describe the PCP of peptide:
  - Hydrophobic moment (M)
  - Hydrophobicity (H)
  - Charge (C)
  - Isoelectric Point (I)
  - Penetration Depth (D)
  - Orientation of Peptides relative to the surface of membrane (O)
  - Propensity to Disordering (R)
  - Linear Moment (L)
  - *In vitro* aggregation (A)
- Each optimized cluster is defined by a subset of these characteristics and intervals of the values of the corresponding characteristics

Results and discussion

Conditions of creation training and test sets for the predictive models

- Positive set is formed on the basis of condition MIC <25 mg/ml
- Negative set is formed on the basis of condition >100 mg/ml
- Sets were performed with the following restrictions:
  - Sequence length 10-16 amino acids
  - Without intra-chain bonds
  - Without unusual amino acids

Results and discussion

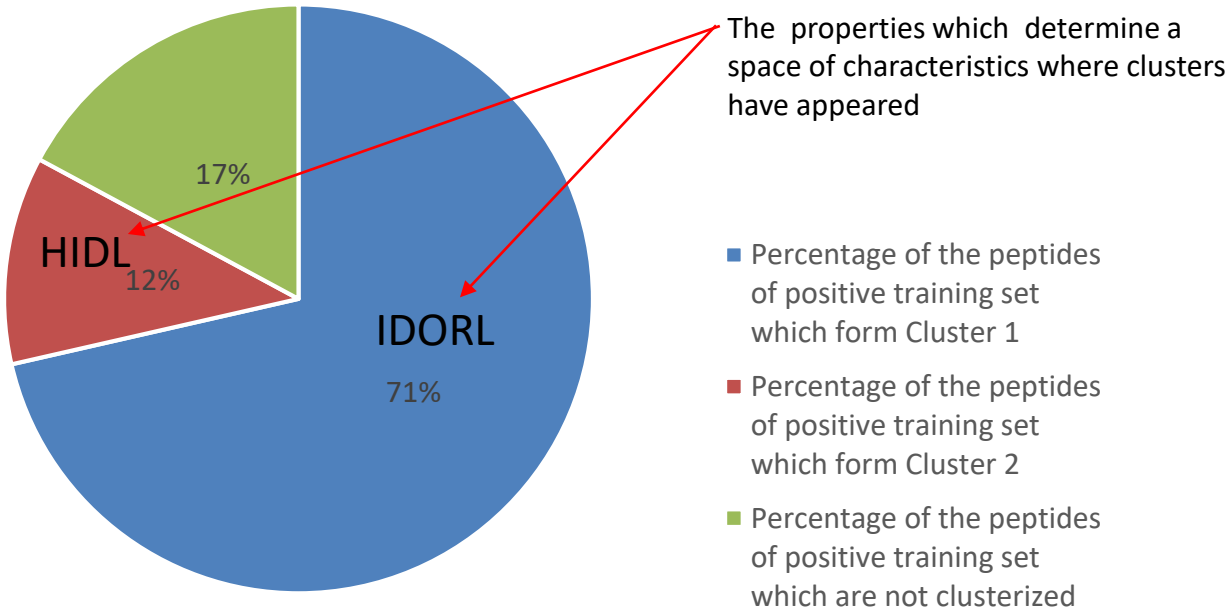
- Detailed description of the algorithm relied on which the predictive model for Escherichia coli ATCC 25922 has been developed, can be found here [2,4]
- Current presentation describes predictive models for some Gram positive (Staphylococcus aureus ATCC 25923 and Bacillus subtilis) bacterial strains and a model for the prediction of hemolytic activity, which are based on the same algorithm as for Escherichia coli ATCC 25922

Results and discussion

Predictive model for Staphylococcus aureus 25923

Optimized clusters obtained for peptides active against Staphylococcus aureus 25923

H – Hydrophobicity
I – Isoelectric Point
D – Penetration Depth
O – Orientation of Peptides relative to the surface of membrane
R – Propensity to Disordering
L – Linear Moment



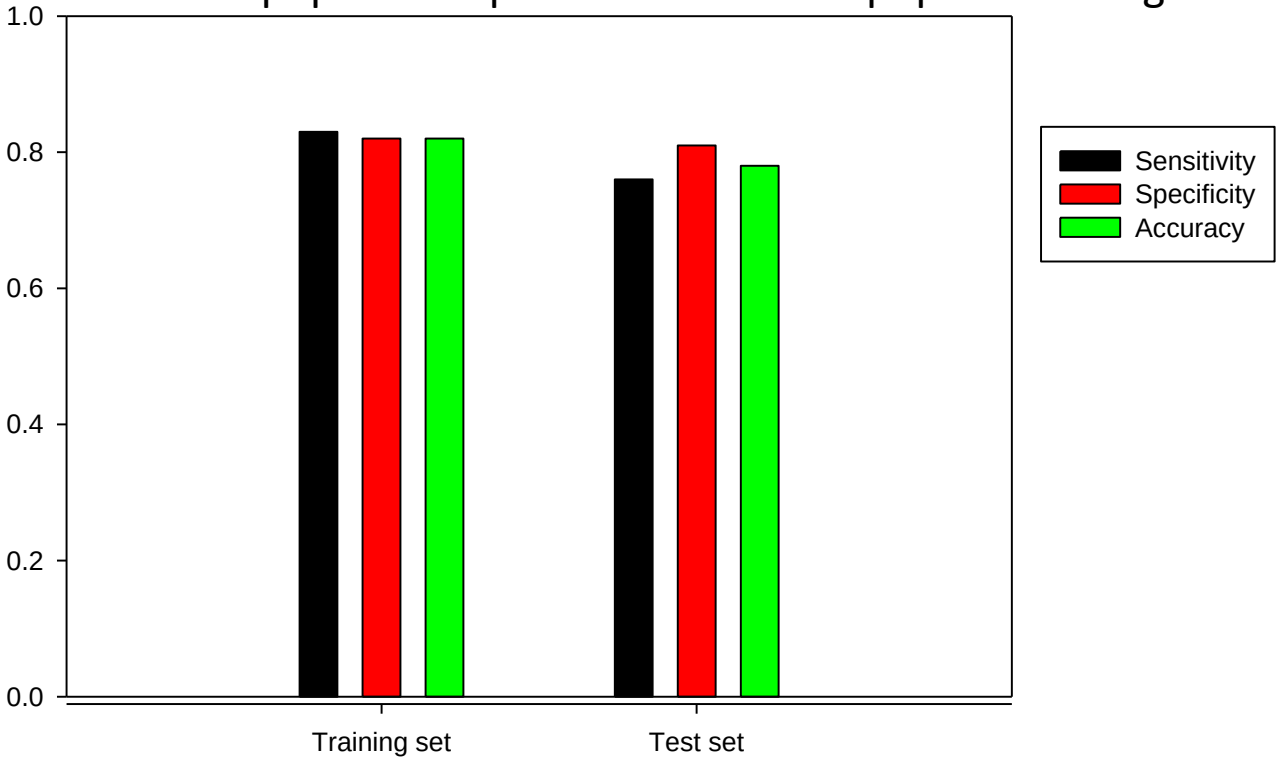
Results and discussion

Predictive model for Staphylococcus aureus 25923

Sensitivity, Specificity and Accuracy for training and test sets

Training set - 140 peptides in positive set and 140 peptides in negative set

Test set – 37 peptides in positive set and 37 peptides in negative set

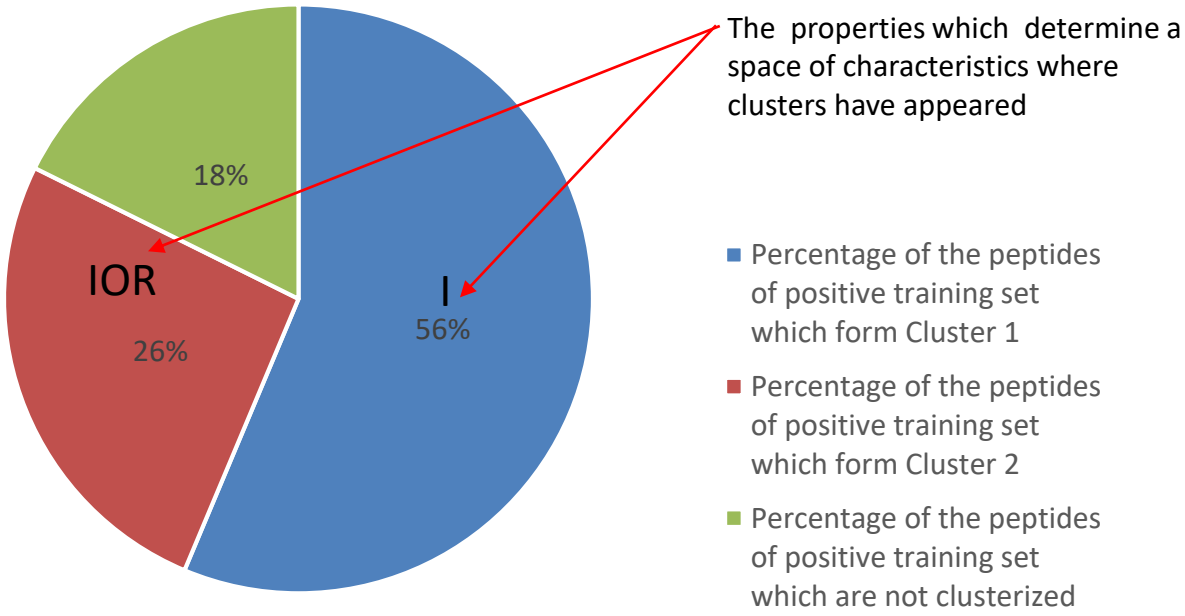


Results and discussion

Predictive model for Bacillus subtilis

Optimized clusters obtained for peptides active against Bacillus subtilis

I – Isoelectric Point
O – Orientation of Peptides relative to the surface of membrane
R – Propensity to Disordering



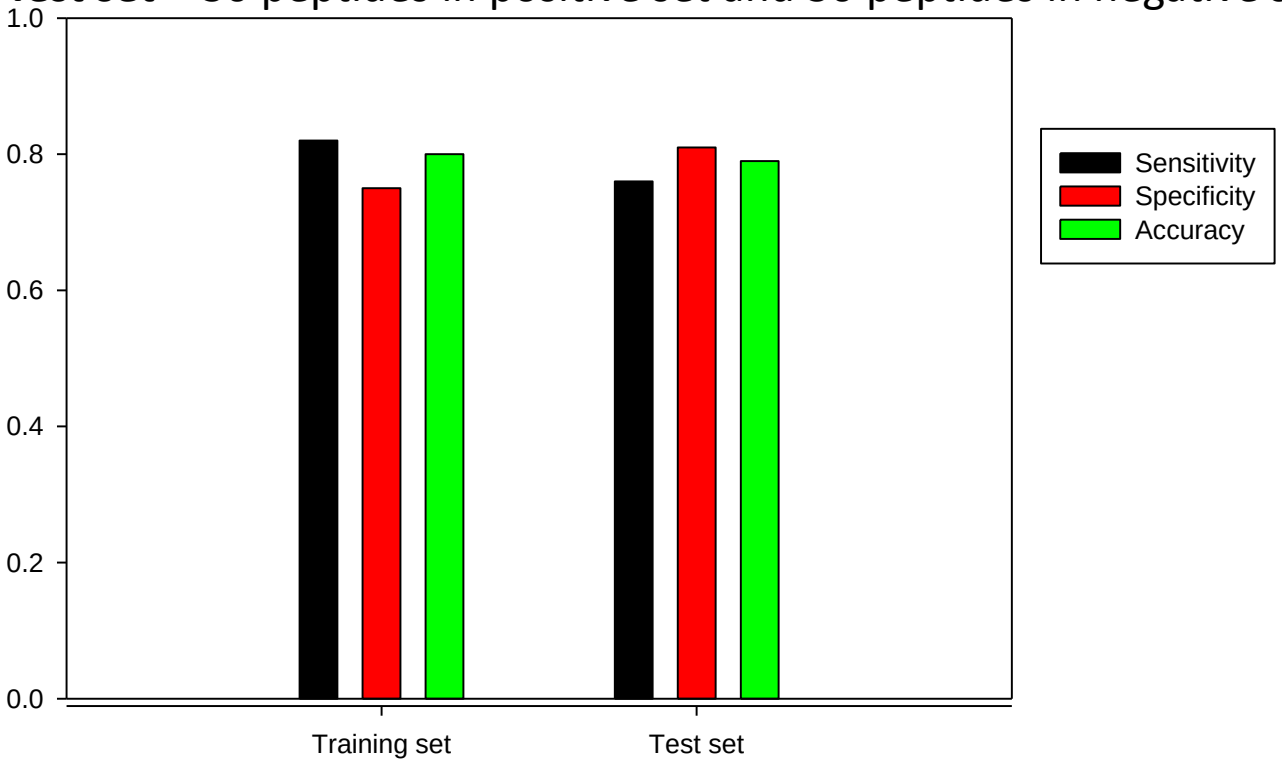
Results and discussion

Predictive model for Bacillus subtilis

Sensitivity, Specificity and Accuracy for training and test sets

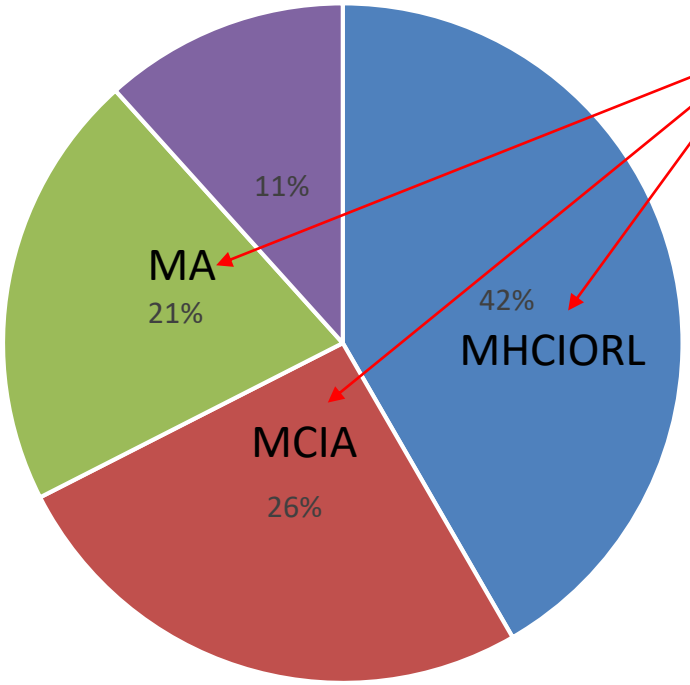
Training set - 100 peptides in positive set and 100 peptides in negative set

Test set – 30 peptides in positive set and 30 peptides in negative set



Predictive model for hemolytic activity prediction

Optimized clusters obtained for peptides non-active against Human erythrocytes



The properties which determine a space of characteristics where clusters have appeared

- Percentage of the peptides of positive training set which form Cluster 1
- Percentage of the peptides of positive training set which form Cluster 2
- Percentage of the peptides of positive training set which form Cluster 3
- Percentage of the peptides of positive training set which are not clusterized

M – Hydrophobic moment
H – Hydrophobicity
C – Charge
I – Isoelectric Point
O – Orientation of Peptides relative to the surface of membrane
R – Propensity to Disordering
L – Linear Moment (L)
A – *In vitro* aggregation (A)

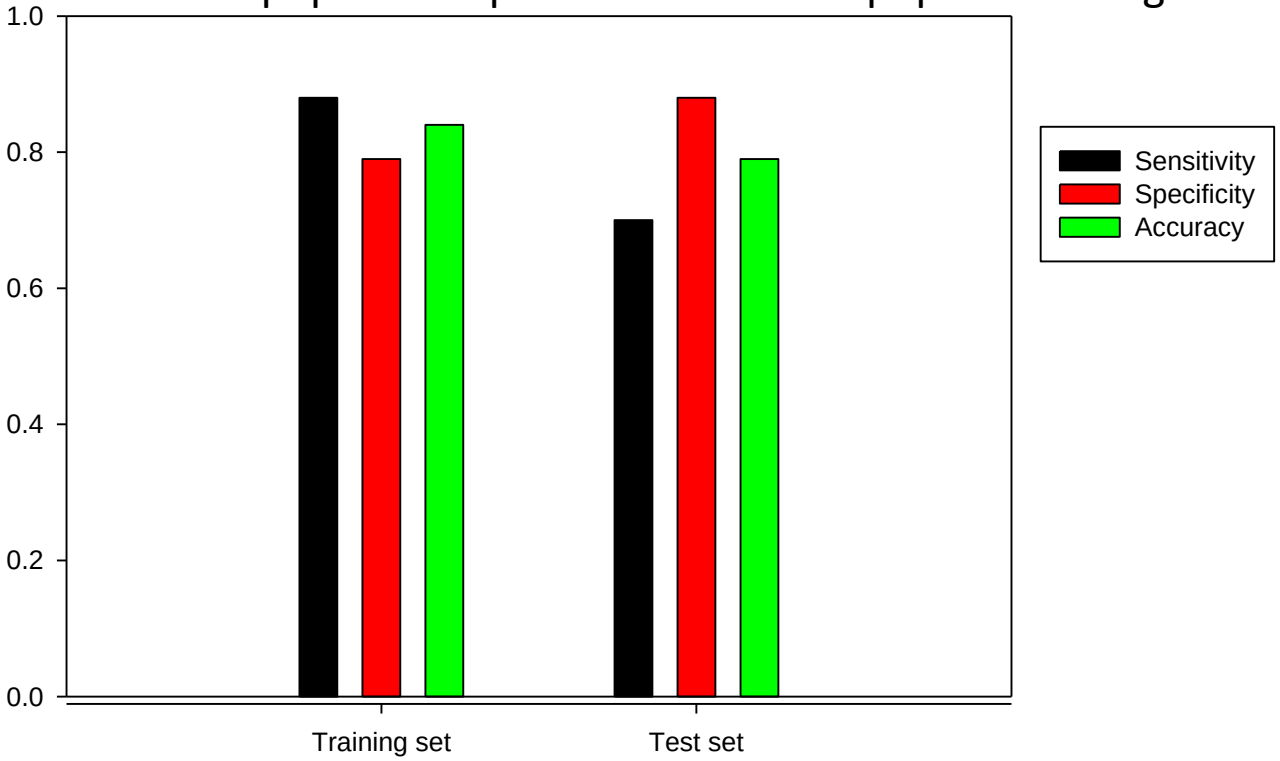
Results and discussion

Predictive model for hemolytic activity prediction

Sensitivity, Specificity and Accuracy for training and test sets

Training set - 120 peptides in positive set and 120 peptides in negative set

Test set – 43 peptides in positive set and 43 peptides in negative set



Results and discussion

Screenshot of SP Page of DBAASP (https://dbaasp.org/prediction)

Paste sequence(s) in FASTA format ('+' can be added to the end of the sequence in the case of C-Terminal amidation) :

```
>1
TASQAEWFKARRWQWRMKKLGA
>2
KLALKLALKALKAALKLA+
>3
AKKVFKRLGIGAVLKVLTTG
```

The results are presented as positive or negative predictive values (PPV and NPV).

Submit

ID	Strain Type	Class	Predictive value
1	Staphylococcus aureus ATCC 25923	Not Active	0.80
1	Bacillus Subtilis	Active	0.81
2	Staphylococcus aureus ATCC 25923	Active	0.82
2	Bacillus Subtilis	Active	0.81
3	Staphylococcus aureus ATCC 25923	Not Active	0.80
3	Bacillus Subtilis	Not Active	0.76

Results and discussion

Peptide design

- Peptides were designed by the following principles:
  - Length of peptides is 13, based on the data of most frequent peptide lengths in DBAASP and requirement of low cost.
  - Sequences were selected from randomly generated amino acid sequences according to the requirements: the values of physical-chemical properties should correspond to statistically more reliable clusters obtained for Escherichia coli ATCC, Staphylococcus aureus 25923, and Human erythrocytes
  - 22 and 12 sequences have been generated as having potential against Escherichia coli ATCC 25922 and Staphylococcus aureus 25923, respectively. 24 sequences have been created as non-active against Human erythrocytes

Results and discussion

- Peptides have been synthesized relying on generated sequences
- Susceptibilities of Escherichia coli ATCC 25922, Staphylococcus aureus 25923 and Human erythrocytes against synthesized peptides were tested *in vitro*
- Peptides were considered as active and non-active against the corresponding bacterial strains if they have MIC<50 and >100 mg/ml correspondingly
- Peptides are considered as non-hemolytic if they have <10% of hemolytic activity of 0.1% Trilton X-100 which is use as reference, at peptides' concentration 100 mg/ml

Results and discussion

In vitro test results

- 21 from 22 predicted peptides show activity against Escherichia coli ATCC 25922
- 10 from 12 predicted peptides show activity against Staphylococcus aureus 25923
- 22 from 24 predicted peptides do not show hemolytic activity.

Conclusions

- **SP tool is available online in DBAASP website (https://dbaasp.org)**
- ***In-silico* test of SP predictive models shows accuracy about 0.8 for Staphylococcus aureus 25923, Bacillus subtilis, and for hemolytic activity prediction.**
- **The tests show that 95% and 80% of the peptides, designed as active against Escherichia coli ATCC 25922 and Staphylococcus aureus correspondingly, show also high antimicrobial activity *in vitro*.**
- **92% of the peptides, *de novo* designed as non-active against Human erythrocytes do not show hemolytic activity *in vitro***
- **SP tool can be satisfactorily used for the development of peptide–based antiinfectives**

Acknowledgments

This work was supported by International Science and Technology Center provided through National Institute of Allergy and Infectious Diseases / National Institutes of Health (G-2102) and Shota Rustaveli National Science Foundation (DI-2016-9)

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