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Scanning, Characterising, Isolation and Testing Hypothesized Antimicrobial Peptide, (Wf24) From Chitin Binding Domain of Halorhabdus Tiamatea

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Abstract – Several studies have shown an increase in resistance to antibiotics. Hence, different approach in which the natural biological sources have been targeted to extract antimicrobial peptides AMPs.

These AMPs should face less resistance by microbes and hence, are considered a novel approach in fighting these microbes. It has been reported by Bormann et al. 1999, that a similar chitin-binding domain has been isolated from Streptomyces Tendae, which possesses antifungal activity. The Purpose has been to scan and characterise the chitin-binding domain from Halorhabdus timatea and to isolate and test the candidate sequence derived from this domain. The sequence has been synthesised by NeoLab. Three servers, CAMP, APD Antibp2, which uses four logarithms, discriminant analysis (DA), Support Vector Machine (SVM), Artificial Neural Network (ANN) and Quantitative Matrices (QM), have been used to predict the antimicrobial activity of the sequence. Phyre2, Swiss-model and Loop servers have been used to predict the secondary structure of the candidate sequence.

The programs used have predicted fairly similar results for the antimicrobial activity of the candidate sequence WF24. Structure prediction programs have predicted similar results, showing an alpha helix toward the N-terminus. The PPM server has shown a full immersion of the amphipathic alpha helix into the membrane.

WF24 resides in the catalytic domain that belongs to glycoside hydrolase family 5. The naturally occurring end-tagging of the sequence WF24 with the hydrophobic residues tryptophan and phenylalanine, and the presence of glycine and (RW) motif within WF24, represent distinctive properties of a lot of AMPs in literature.

Due to the limited time frame, WF24 has been tested against only 10 types of microbes, which decreases the chances of revealing antimicrobial activity. No positive results have been found. Because of the several advantages of WF24, further investigation using WF24, in its current form against several other types of microbes, should be performed.

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INTRODUCTION

Contagious diseases are the third most common cause of death in developed countries, in spite of accessibility to antibiotics (Nathan, 2004). Though, the control of some bacterial infections has become

complex issue due to the escalation of bacterial resistance against traditional antibiotics.

There are about 50 amino acids present within peptides which contain a positive charge. AMPs show a wide range of antimicrobial activity that is comparable to peptides that occur naturally, and as a result, these bacteria illustrates relatively low levels of resistance to AMPs (Pathak & Chauhan, 2011; Hancock & Chapple, 1999).

At present, more than 2,426 AMPs have been recorded in the ADP, Antimicrobial database (Wang & Wang, 2004). The majority of them are cationic peptides, with only a small number being anionic, and so demonstrate the capacity to fold in to amphipathic conformations when in contact with the membranes (Brogden, 2005).Lannucci et al. (2011) reported that the secondary structure of AMPs falls into four main structures: α-helices, β-strands, cyclic, and extended peptides.

The primary asset of AMPs are their mechanisms of action (Feder et al., 2000), which involves the disturbance of the integrity of the bacterial membrane and targeting certain cytoplasmic contents. This mechanism occurs by binding to membrane and then by membrane permeation/insertion, (Lannucci et al., 2011).

AMPs demonstrate a unique mode of action, and so are thought to be strong candidates for use as anti-infective compounds. Numerous AMPs can not only destroy bacteria, but can simultaneously neutralise the toxic effects of LPS (Mangoni & Shai, 2009). LPS is known to effect septic shock, which makes this neutralisation ability of AMP unique when contrasted with traditional antibiotics.

The use of synthetic AMPs also means that unnatural amino acids can be included during the synthesis, and such alterations can lead to the peptide showing greater levels of stability to proteolysis. This modification has been shown in some studies to have augmented antimicrobial efficacy to levels acceptable for possible clinical application (Fernandez-Lopez et al., 2001; Reddy et al., 2004).

A computational approach can speed up the development of drug design and discovery. At present, the majority of studies has emphasised the use of Insilico modelling and screening of new AMPs. As a result, bioinformatic techniques have been devised to predict new AMPs (Wang et al., 2011).

The scope of this study has been a chitin-binding domain that has been isolated from the halophile *Halorhabdus timatea.*It has been hypothesised that the candidate sequence (WF24), extracted from a chitin binding domain, will have a hydrolase-like antimicrobial activity, by which it acts mainly against the carbohydrates/polysaccharides within the bacterial membrane, consequently the membrane will lose its rigidity leading to bacterial cell fatality.

Halorhabdus tiamatea belong to the archaeal domain, and are phylogenetically separate from both eukaryotic cells and bacteria. They are fairly heat stable, and can withstand temperatures between 55-60°C. They are strong halophiles that show optimal growth at high levels of salinity (3.4–5.1M NaCl). They have been shown to have a high ratio of acidic to basic amino acids by genome sequencing and proteome analysis, and this characteristic is probably needed for protein activity at high salt levels (DasSarma & DasSarma, 2012).

Halorhabdus tiamatea encodes proteins that are features required for thermophiles and enzymes, which require light. This provides evidence that this species holds a unique position as a polysaccharide degrader in climates with extreme salinity. In total, *Halorhabdus tiamatea* has 50 glycoside hydrolase genes (Werner et al., 2014).

MATERIALS AND METHODS

MATERIALS

Peptide synthesis

The sequence WF24 has been synthesized and supplied by NeoLab and presented in a powder form weighing 10 grams. 5µl. To purify the peptide and confirm its identity, HPLC and Mass Spectrometry have been used, respectively.

Lab materials

Dimethyl sulfoxide DMSO (manufacture's reference, D5879) has been supplied by Sigma-Aldrich. Brain heart infusion, tryptone soya and sabouraud dextrose agar (manufacture's references, CM1135, CM0131 and CM0041, respectively) has been supplied by Oxoid in the following concentrations, 37, 40 and 65 g/l, respectively. Moreover, M.R.S and nutrient (manufacture's reference, CM0361 and CM003, respectively) have been supplied by Oxoid in the following concentrations 62 and 28 g/l, respectively.Antibiotic chloramphenicol discs containing 25micro gram (type M14) has been supplied by Mast-Diagnostics.

Micro-organisms used

The sequence was tested against four types of gram positive bacteria, Staphylococcus aureus, Bacillus cereus, Staphylococcus epidermidis and Streptococcus pyogenes, have been supplied by NCTC and their manufacture's reference has been 10788, 9946, 7474 and 8198, respectively. Three

types of gram negative bacteria, Escherichia coli, pseudomonas aeruginosa and Salmonella enterica, have been supplied by NCTC (National Collection of Type Cultures) and their manufacture's references have been 8196 and 6749, respectively. Three fungi Candida albicans, Candida glabrata and Candida tropicalis have been supplied by NCYC (National Collection of Yeast Cultures) and their manufacture's references have been 597, 350 and 1503, respectively.

METHODS

Computer based methods:

Overall, three servers, CAMP, APD Antibp2, which uses four logarithms, discriminant analysis (DA), Support Vector Machine (SVM), Artificial Neural Network (ANN) and Quantitative Matrices (QM), have been used to predict the antimicrobial activity of the sequence. Phyre2, Swiss-model and Loop servers have been used to predict the secondary structure of the candidate sequence.InterProScan5 server was used to show hits, families, and domains within protein sequences.PROTPARAM serve was used for physiochemical characteristics of protein sequences. The mechanism that the CAMP program uses to predict antimicrobial activity is a systematic approach. The parts that were obtained from the DA program were then analyzed individually using APD program. Parts obtained from DA of the CAMP databases were analyzed using antibp2 for comparison with the SVM logarithm in order to measure the impact of all residues within the sequence.Phyre2 server was used for structure prediction of the sequence. Loop server was used for structure prediction.

Lab based method

5mg of the powder WF24 have been dissolved in 1000 µl of deionized sterile water then shake using a vortex. To aid the dissolution process 300 µl of DMSO have been added to the solution, 100 µl at a time whilst shaking. The final concentration of the solution has been 3.84 mg/ml.

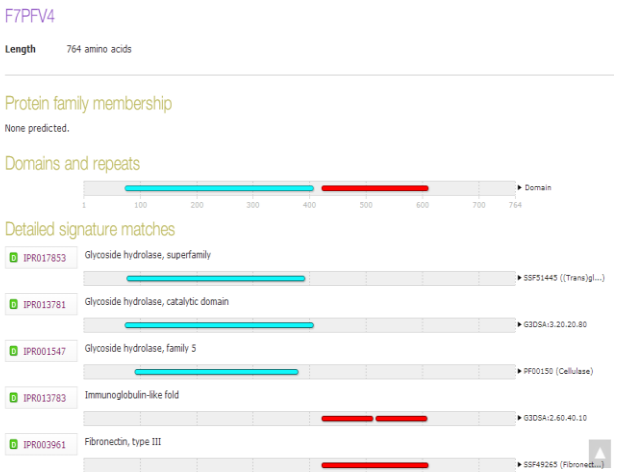
The second concentration to be used has been 5.71 mg/ml resulting from dissolving 2mg of WF24 in 175µl of DMSO and 175µl of deionized sterile water.

Both of the concentrations mentioned above have been tested against 10 microbes. 10 µl of each solution have been dispensed, in triplicates, against the relevant petri dish. Hence, 30 petri dishes have been used for each concentration. In addition, two different negative controls have been used. For the first solution, the negative control has been formulated using 1ml of deionized sterile water and 300µl of DMSO. For the second solution, the negative control has been formulated using 50µ of deionized sterile water and 50µl of DMSO.

RESULTS

Computer based result:

InterProScan5 results



* Figure (1) above shows results of analysing the whole chitin-binding domain, in particular, its relevant families and specific domains.

The first area is the glycoside hydrolase superfamily (IPR017853), which extends from residue 75 to 405 and contains glycoside Hydrolase, catalytic domain (IPR013781) and glycoside hydrolase family 5 (IPR001547).

In addition, it contains a fibronectin, type III (IPR003961), which extends from residue 422 to 608; here a signal sequence from 1 to 75 is called a twin-arginine translocation (Tat)

APD results

The outputs and results acquired from processing the chitin binding domain sequence using the APD program has shown no clear concrete evidence suggesting that these parts carry effectiveness against any microbes. Instead, overall measurements have shown the percentage of different amino acids, the net charge, and hydrophobicity percentage within each sequence. All sections have shown a high ratio of hydrophobicity ranging between 21 to 41 %. Conversely, most of the analysed sequences have shown a negative net charge.

The parts that were obtained from the DA program were analyzed individually using the APD program. The first part illustrated 50% hydrophobicity and a zero net charge. Furthermore, upon comparison of the sequences within the list of existing antimicrobial peptides in the database, the highest similarity to the input part I was 44.44% with AP00965, which is dermaseptin-C3 of a South American frog.

The second part illustrated 55% hydrophobicity and a zero net charge. A comparison of the sequence with the database has shown highest similarity of 44.33% to an existing antibiotic named Maculatin 1.4, referred to as AP00770 in the database, extracted from the frog Litoria eucnemis of Australia.

The third part indicated 40% hydrophobicity and one net negative charge. A sequence comparison to the database has shown highest similarity of 43.47% to an existing antibiotic named CPF-C1 caerulein precursor fragment-C1, referred to as AP01695 in the database, which is extracted from the Eritrea clawed frog, Xenopus clivii.

The fourth part demonstrated 50% hydrophobicity and a zero net charge. A sequence comparison to the database has shown highest similarity of 43.47% to an existing antibiotic named Plasticin-DA1, referred to as AP01385 in the database, this is extracted from Pachymedusa dacnicolor (PD), North America.

Similarity with seq	APD ID	Name	Length	Net charge	Hydrophobic Residue %	Sequence
44.44% with (SG22)	AP00965	Dermaseptin-C3	27	1	51%	SVLSTITDMAKAAGRAALNAITGLVNQ
43.33% with (TL29)	AP00770	Maculatin 1.4	21	1	52%	GLLGLLGSVVSHVLPAITQHL
43.47%with (GI20)	AP01695	CPF-C1	17	3	52%	GFGSLLGKALRLGANVL
44.44% with (WF24)	AP01385	Plasticin-DA1	23	0	43%	GVVTDLLNTAGGLLGNLVGSLSG

Data above obtained from APD**Table (1) above shows percentage of similarity between the four parts derived from the chitin-binding domain and the sequences available within the server.

PROTPARAM RESULTS

Full analysis of the complete chitin binding domain using PROTPARAM indicated that the aliphatic index was 69.14% and that the majority of chitin binding domain sequence possess negative charges. In addition, the total number of negatively charged residues is 130 (approximately 17%), compared to 39 positively charged residues (approximately 5%).

Antibp2 results

In this first section of analysis, no positive results were found for the full sequence using the N and C-terminals based method, nor the 'N terminal' based method. However, using the C-terminal based method demonstrated some effectiveness against microbes, with a score of 0.534. This result was acquired from analyzing amino acid residues, starting from residues 8-23 (GLGALGAAATTQLFS) within part SG22.

With TL29, a positive result was acquired when using the full sequence composition based method for the sequence TAAAGLAGLGVGSGVVGSAAAAGIPTPWL. This had a score of 0.702. Moreover, using the N-terminal based method a positive result was obtained with a score of 0.675. This result was acquired from analysis of amino acid residues starting from residues 4-19 (AGLAGLGVGSGVVGS).

GI20 was also analyzed but did not provide a positive result using the full sequence composition, nor with the N and C-terminals based methods. By contrast, using the N-terminal based method a positive result was recorded, at 0.615. This result was acquired from analysis of amino acid residues starting from residue No. 6 to residue No. 20 (DPEGNKVILRGVNVI). Another sequence within GI20 demonstrated a positive score of 0.035 from analysis of amino acid residues starting from residues 1-16 (GNLLRDPEGNKVILR).

Similar to GI20, WF24 was also analysed, but did not provide a positive result using the full sequence composition, nor with the N and C-terminals based methods. Interestingly, using the N-terminal based method resulted in a positive score of 0.769 for potential activity of the sequence against microbes. This result was acquired from analysis of amino acid residues starting from residue No. 2 to residue No. 16 (VDLIREHALRNLIVI) within WF24. Another sequence within WF24 has shown a positive score of 0.199 from analysis of amino acid residues starting from residue 8-22 (HALRNLIVIGSPRWS). *

Swiss-model result

The Swiss-model (Arnold et al. 2006) was used to investigate sequence part WF24; it showed results of secondary structure prediction, but with no hits. The results also obtained from Swiss-model server indicate that sequence of WF24 shows a presence of an alpha helix from residues 2-12 (VDLIREHALRN), as well as the beta extend from 13-16 (LIVI).

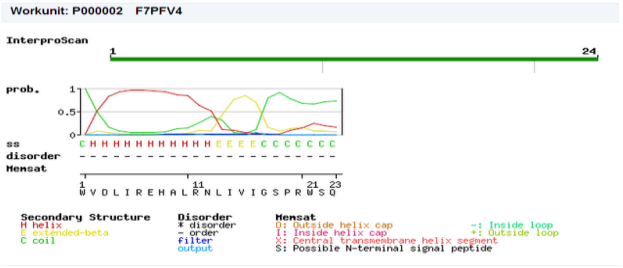


Figure (2) above shows the location of the alpha helix and beta conformations within the candidate sequence WF24

Phyre2 result

In addition, the phyre2 server, founded by Kelley and Sternberg (2009) was used to analyse the full-

length of chitin binding domain as well as the candidate sequence, and the fourth part WF24. As shown in figure (4), with less than 55% model reliability, there is a probability of existence of an alpha helix that stretches from residues 2 to 10 as well as a beta structure that extends from residues 13 to 16 (LIVI).

Protein Homology/analogy Recognition Engine

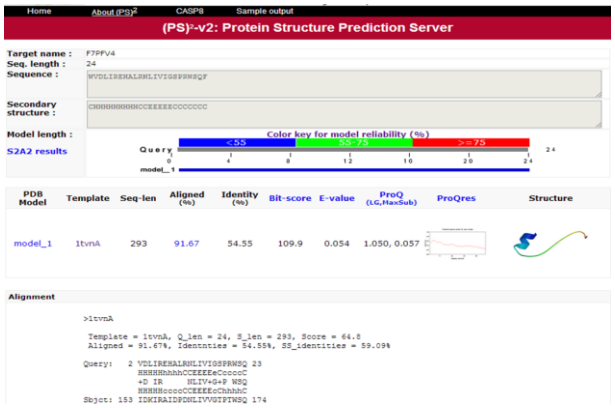


***Figure (3) above shows the location of the alpha helix and beta conformations within the candidate sequence WF24.**

Based on the results shown in figure (3), comparison between 1TVNA and WF24 has shown high percentage of similarity in identity which was 54.55%. it also shows that the alpha helix stretches from residues 2 to 10 whilst the beta helix extends from 13-16 (LIVI) within both of the sequences.

Template	Aligned (%)	Identity (%)	E-value	Classification:	Molecule:	Fragment:
1tvnA	91.67	54.55	0.054	Hydrolase	cellulase	catalytic domain
1g0cA	91.67	45.45	0.14	Hydrolase	endoglucanase	alkaline cellulase k catalytic domain
1egzA	91.67	45.45	0.21	Hydrolase	endoglucanasez	catalytic domain

*Table (2) shows results obtained from phyre2 server. As shown, 1tvnA, 1g0cA, and 1egzA were the models that matched the candidate sequence WF24 with highest percentage of similarity.

```
      PWVDLIREHALRNLIVIGSPRWSQF
      CCHHHHHHHHCCCCEEEECCCCCCCC
      645666677678747765776566 6
END_SECTION

SECTION_SS_PROBABILITIES
Secondary structure prediction

Output format is the following:
1st line -> query sequence
2nd line -> probability for helix
3rd line -> probability for beta strand
4rd line -> probability for coil
```

*Figure (4) shows results obtained from the Loop server. The number shown at the bottom of each letter of the sequence, represent the probability of formation of the relevant secondary structure on a scale from 1 to 10, 1 being the lowest and 10 the highest probability.

Loop result:

The loop result above illustrates that the alpha helix starts from valine and ends at histidine, stretching from amino residues 2 to 10, whilst beta section starts from Leucine and ends at glycine, residues 13 to 16 (LIVI). The beta related result supports those acquired from phyre2 and the Swiss-model.

Lab based results:

The 10 microorganisms that have been tested did not show significant results.

DISCUSSION

Halorhabdus

Since the amino acid chain has been taken from the organism labelled *Halorhabdus tiamatea*, the sequence was expected to own the same properties, which the organism was encoded for, as clarified by Werner et al. (2014).On the other hand, the part being deducted from the sequence may lack the properties stated above; consequently, the properties expected to emerge from *Halorhabdus tiamatea*, being resistant to high temperature and salinity, may be lost.

This implies that the different functions of the residues may be affected by presence of other residues within the full sequence, while the absence of these residues may lead to loss of a particular property. Overall, this organism bears many qualities that make its protein sequence an interesting material for further research.

The study performed by Werner et al. (2014) on genetic sequences showed that all of the 50 glycoside hydrolase genes have ability to degrade carbohydrates externally. Thus, this organism can utilise plant polysaccharides efficiently

Computer based

InterProScan5

After use of InterProScan 5, founded by Jones et al. (2014), as another way to analyse the entire sequence and to detect the presence of domains, families and important sites, the sequence were found to contain the following properties. These results justify the selection of the candidate sequence.

Signal sequence from residues 1 to 75 is called a twin-arginine translocation (Tat) as described by

interproscan5. This has given an indication that this enzyme is extracellularly excreted.

The sequence analysis produced from the CAMP results indicated that the WF24 is contained in the area extending from residues 75 to 405, which might be responsible for the metabolism of carbohydrates.

SG21 extended from 49-70, this is not within the glycoside hydrolase superfamily domain; in fact it is completely within the signal sequence of the TAT.

TL29 extended from amino acids 54 to 82. This implies that it starts in the signal sequence and continues into the beginning of glycoside hydrolase superfamily domain.

GI20 extended from amino acids 86 to 105. Hence, it starts at the very beginning of the sequence of glycoside hydrolase superfamily domain. This part (G120) was not preferred over the selected part (WF24), which lies in the center of the hydrolase catalytic domain.

CAMP

Not a single amino acid has been neglected , as each amino acid in the sequence has a distinctive physiochemical property that may implicate a significant role on the sequence to acquire antimicrobial activity. SVM, RF classifier, ANN and DA programs were used. Even though the mentioned programs used had different logarithms, the analysis results were fairly similar.

The antimicrobial activity probability using the mentioned programs was calculated using the following logarithms DA, RF and SVM. The calculation of effectiveness against microbes resulted in a range from 0.0 to 1.0.

As the results were approximately identical in all four logarithms used, DA was adopted. Furthermore, efficient parts within the domain that had the highest probability, higher than 0.9, were selected and cropped, whilst the rest of the sequence that had a probability of less than 0.9 were neglected. At this stage, as the results are based on computer programs, they were not validated with real live tests. Thus to produce further credibility, another program (ADP) was used.

Based on results obtained from CAMP, APD and AntiBp2, it was decided to rely on the DA logarithm from the CAMP server. DA results were picked as the DA results mostly agree with results from other programs, and that the DA selected parts were mostly in the length range of twenty amino acid.

PROTPARAM

The protopram results have given the impression that it is not expected to easy find frequent sites carrying positive charges along the chitin binding domain within the same vicinity. Moreover, the PROTPARAM results mentioned above implicate a high hydrophobicity of residues being analysed within any selected sequence.

APD

Unlike other databases that were used to search for antimicrobial peptide activity, the researcher, using this APD database, needed to manually enter a number of peptide sequence that were less than 200 amino acids, as the database would not accept it otherwise.

As compared to other parts, the highest percentage of hydrophobicity was not the best deciding factor for likelihood of the sequence being AMP (WF24). For example, even though the first part (SG22) had the highest hydrophobicity of 55%, it was not selected. This can be justified by the fact that the high percentage of hydrophobicity might be related to cytotoxicity as reported by Pasupuleti et al. (2009). This causes a contrary effect that will influence the host cells instead of acting as antimicrobial peptide.

The peptides that possess a higher percentage of hydrophobicity are usually able predicted to form and configure in an alpha helix motif (Wang & Wang, 2004). Therefore, depending on that, the fourth part (WF24) contains 12 hydrophobic residues, 8 of which are on the same side, giving it a high probability to form the amphipathic alpha helix. As these 8 amino acids represent a third of the total fourth part (WF24) in an alpha-helical pattern, it is almost similar to other parts. For instance, there were 8 residues in part one (SG22) representing 36.36%, 9 in part two (TL29) which represents 31% and only 4 in part three (GI20), which represents 20% of their total length, respectively. By comparing the probability of all parts to form an alpha helix inside every part, the result was positive for parts one (SG22) and four (WF24), with the latter surpassing the former with two net positive charges.

Antibp2

As observed from the results, part one has shown positive results when it was processed using the C-terminal based method. By contrast all other parts have shown positive results when processed using the 'N Terminal' based method.

This means that in case of scanning a sequence that is longer than 15 amino acids, for instance WF24, the program will complete tasks by

processing the sequence in the form of multiple templates, each of which strictly consists of 15 amino acids. This is due to the window for the program is programmed in a manner that uses models of 15 residues; this is based on the observation that approximately 60% of the antibacterial peptides have a sequence length in the range of 20-30 residues, as reported by Lata et al., (2010).

Upon comparison of all the results acquired from the antiBp2 server, using the four methods of analysis, N-terminal, N and C-terminal, C-terminal and the full sequence composition based methods. It has been noted that the second part (TL29) had a fairly high score (0.702), although, the fourth part (WF24) remains the best candidate, since it carried the highest score of 0.769.

Swiss-model

The Swiss-model program, designed by Arnold et al. (2006), was used to predict the secondary structure of the sequence WF24. The program did not show any hits, this is probably due to the short length of the sequence. Moreover, the results that were obtained from Swiss-model server indicated that the sequence of WF24 possesses an alpha helix within the residues 2-12 (VDLIREHALRN), as well as, a beta structure that extends from residues 13-16 (LIVI).

Phyre2 server

The Phyre2 server demonstrated almost the same result obtained from Swiss-model. The models 1tvnA, 1g0cA, and 1egzA have been matching the candidate sequence, WF24, with highest percentage of similarity.

These results have been considered very reliable for more than one reason. The three templates that have shown high resemblance in identity, compared to the candidate sequence WF24, are actually classified as hydrolases. Likewise, the sequence WF24 is a chitin binding domain that is a hydrolase which targets polysaccharides, a fact that strengthens the phyre2 results. This resemblance supports the hypothesis mentioned previously.

Loop discussion

The Loop server has shown identical results to those obtained from phyre2 server. On the other hand, there has been a slight difference upon comparing location of alpha helix in both loop and Swiss-model. As Swiss model has shown an additional 2 residues within the alpha helix conformation to extend from residue number 2 to 12.

Comprehensive discussion about (WF24)

Those databases mainly consists of two sets of data that will aid researchers finding candidate antimicrobial peptides. The first set is a collection of antimicrobial sequences that were established in vitro and have a known activity, whilst second set of data is actually discovered insilico which were reported in the literature earlier, as mentioned by Thomas et al. (2010); it illustrates parts that may contain effective antimicrobial residues. The physiochemical properties were incorporated into a database to build a server that could be used to search for antimicrobial activity based on those physiochemical characteristics.

Moreover, Porto et al. (2012) suggested that these methods of prediction and databases can be much more accurate and reliable if they strictly contain AMPs that have been practically, in vitro, validated against at least one type of microbe.

It is noteworthy that all of the previously mentioned methods do not possess an ability to determine a feature that can reasonably interpret and/or precisely predict any biological implication, as mentioned by Wang et al. (2011).

The existence of different servers has shown that each system possesses weaknesses and strengths. Therefore, there is no one single reliable server that can be relied on to predict protein structure as mentioned by Kelley and Sternberg (2009).

Such sophisticated tools are essential in any investigation into AMPs, as they can speed up the search process and reduce number of candidate peptides that need to be clarified (Brahmachary et al., 2004).

There are two crucial features that distinguish antimicrobial peptides and aid them in their association to microbial membranes; one is the net charge, while the other is the hydrophobicity, as reported by Gopal et al. (2014).

Hancock (2006) suggested that in order to successfully attract the negatively charged microbial cell membrane, the AMP should have a positive net charge ranging from 2+ to 8+. Moreover, the relatively high hydrophobic residues content within the antimicrobial peptide will result in high hydrophobicity, ideally ranging from 40 to 60%, which will enable the AMP to vigorously penetrate the microbial cell membrane, as explained by Zelezetsky and Tossi (2006).

To clarify, in the overall strategies adapted in the selection process, there were two main factors that were considered before a selection was made, these being hydrophobicity and the net positive

charge. These are two strategies out of several available that were explained by Maccari et al. (2013) in order to select a candidate sequence. The main purpose of this strategy was to make the sequence feature a level of selectivity to microbial cells rather than host cells.

It has been reported by Juretić et al. (2012) that both selectivity and biological activity of AMP are associated with the N-terminal more than the C-terminal within the alpha helix conformation.

In this study, an amphipathic helical structure for the sequence has been found. This finding has been based on the results obtained from several structure prediction programs including the Swiss-model, phyre2 and loop programs.

In addition to the diverse content of the residues that makes (WF24) a remarkable candidate, (Pasupuleti et al. 2009) investigated the advantages of using hydrophobic amino acids, for instance, W or F, to end-tag an antimicrobial peptide and his finding has been significant to this study. The investigation illustrated that the mentioned end-tagging process can significantly enhance the antimicrobial activity. In relation to the candidate sequence for this study (WF24), this is a significant advantage that applies to the candidate sequence, which starts with the hydrophobic residues tryptophan (W) and ends with phenylalanine (F) without any intervention.

During this study, the Swiss-model, phyre2, and loop programs demonstrated that arginine-tryptophan (RW) motif does not reside within the secondary structure of the sequence WF24. Nevertheless, this does not nullify the importance of the mentioned motif. In fact, the RW motif will have its impact on antimicrobial activity, biocompatibility and salinity resistance, even if it falls outside the secondary structure, as explained in the Quantitative Structure Activity Relationship study by Saravanan et al. (2014)

Wang et al. 2011 reported that histidine, cysteine, tryptophan and arginine are abundant in antimicrobial peptides and Tryptophan is favored in the protein-membrane interface and Crucial in lipid binding (Wang et al. 2011).

The fourth part (WF24) has been selected since it carries two positive net charge and this increases the probability of connection and adherence of the sequence to the bacterial membrane. In addition, most of the ideal antimicrobial peptides possesses 2 positive net charge or more as reported by (Mukesh Pasupuleti, Artur Schmidtchen et al. 2012)

Lab based discussion

Peptide Self-association

It was demonstrated in this study that amphipathic AMPs containing α-helices are directly linked with three major factors, which are decisive to the activity of the AMPs. These factors agrees with those reported by Jiang et al. (2011). The first factor is the quantity and order of positive amino acids on the peptide's polar side. On the opposite, non-polar side, the quantity and order of hydrophobic amino acids, along with the hydrophobe type, represent another major factor that influences the activity. The last of these decisive factors is the quantity and order of the 'specificity determinants', which are also situated on the non-polar side.

The mechanism by which the second factor directly influences the antimicrobial activity of a given peptide has been explained by the previous work of Jiang et al. (2011). There is an optimum hydrophobicity that should reside on the non-polar side in order for the AMP to achieve maximum antimicrobial activity. Once the optimum hydrophobicity has been exceeded, there will be a great loss in antimicrobial activity.

Results obtained from investigation carried out by Lannucci et al. (2011) indicated that the tyrosin and lysine were potentially more stable than the tryptophan and arginine.

FUTURE STUDY

It is noteworthy that, in case Insilco programs have predicted promising antimicrobial activity for a given sequence, which simultaneously possesses high hydrophobicity that can potentially be toxic to mammalian cells. The sequence should not be aborted, instead a different approach can be considered.

Pasupuleti et al. (2012) reported that a lot of the AMPs available in nature require modification to improve their hydrophobicity, and/or a positive net charge so that their antimicrobial activity can be enhanced. This should be considered during future studies for this sequence.

If the candidate AMP has been experimentally proved to cause mammalian cells toxicity, the AMP should not be indicated for oral or parenteral administration, instead, it should be considered for a topical application. This is recommended, especially if the AMP activity is against fungus, and a lot of antifungal medications are directed for topical application. By producing it in a topical form, direct introduction into circulation through injection will be avoided. This feature will firstly minimize the toxic effect of medication on other organs or non-target tissues. Secondly, this approach will diminish

the hemolytic effect on red blood cells. Thirdly, the possibility of the peptide sequence being cleaved by proteolytic enzymes in blood circulation will be abolished. Finally, renal clearance of AMP from the body will not take place; hence, its duration of action will be increased.

CONCLUSION

To conclude, all of the program used within this project, including 4 different search logarithms, have predicted fairly similar results for the antimicrobial activity of the candidate sequence WF24. On the other hand, all of the program used within the project have predicted similar results for the structure of the candidate sequence (WF24), which has shown an alpha helix conformation toward the N-terminus. In addition, the PPM web server for positioning of proteins in membrane, has shown a full immersion of the amphipathic alpha helix into the biological membrane.

All of the insilico results have shown that the alpha helix is located on the N terminus not the C terminus, a fact that indicates higher biological activity. Moreover, the naturally occurring end-tagging of the sequence WF24 with the hydrophobic residues tryptophan (W) and phenylalanine (F) at N and C terminals, and the presence of both the glycine (G) and the RW motif within the same sequence (WF24), represent distinctive properties of a lot of AMPs in literature.

Unfortunately, no positive results have been found. This might be due the high hydrophobicity of the candidate sequence which leads to occurrence of oligomerization, in particular, upon contact with water. In turn, oligomerization has led to self-association, which can be irreversible and has hindered the alpha helix from reaching its target within the membrane. At this point, the use of DMSO did not completely dissolve the WF24 powder and the solution remained turbid.

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