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In Silico Structural and Functional Analysis of Drug and Vaccine Candidates for *Streptococcus pnuemoniae*

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ABSTRACT

Streptococcus pneumoniae is Gram positive bacterial human pathogen that colonizes the upper respiratory tract and causes life treating diseases such as pneumoniae, bacteriemia and meningitis throughout the world. The disease rates are particularly high in young children, the elderly and patients with predisposing conditions such as asplenia, chronic medical conditions or immunosuppressive illness particularly AIDS. The infection is killing 16 Lakh children less than 5 year, more than 3.7 Lakh in India alone. An improved treatment and vaccine against *S. pneumoniae* is one of the vaccine priorities in the world. The main purpose of this investigation is to study the *In silico* characterization of antigenic proteins involved in disease. Pneumolysin is an antigenic enzyme in against *S. pneumoniae* causing cytalytic at high concentration and cytotoxic at lower concentration which inhibits the capillary movement. Primary protein sequence analysis of pneumolysin was carried out using Protparam tool. SOPMA was used for the prediction of secondary structure of protein. Swiss model was used to predict the 3D structure from X-ray crystal structure (chain A: 3HVN) as a template. The model quality was checked using PROCHECK.. Thiol_cytolysin domain was identified using Pfam. Pneumolysin serves as potential drug and vaccine target for treatment of *S. pneumoniae.*

Keywords: *Streptococcus pneumoniae*, drug, vaccine, pneumolysin

[I] INTRODUCTION

Streptococcus pneumoniae is gram positive bacteria in the shape of slightly pointed cocci. They are usually found in pairs (diplococci), but are also found single and in short chains. *S. pneumoniae* are alpha hemolytic. Individual bacteria are between 0.5 and 1.25 micrometers in diameter. *S. pneumoniae* do not form spores and are non-motile, though they sometimes have pili used for adherence [12]. *Streptococcus pneumoniae* is known to cause bacteremia, otitis media, and meningitis in humans, though it is best known for causing pneumonia, a disease of the upper respiratory tract that causes illness and death all over the world [12]. The pathogenicity of pneumococci has been attributed to various structures, most of which are situated on its surface. The pathogenicity of pneumococci has been attributed to various structures, most of which are situated on its surface. The high morbidity and mortality caused by this micro organism are still, however, poorly understood [11]. One group of factors, such as the capsule and a recently identified protein, provides resistance to phagocytosis and thus promotes the escape of pneumococci from the host immune defense. Other factors, including cell wall

components and the intracellular toxin pneumolysin are involved mainly in the inflammation caused by infection. The inflammation process probably fully develops only after lysis of bacteria by autolysin. Since inflammation is thought to induce most of the symptoms of pneumococcal disease [6, 4].

This group of virulence factors may thus be more directly responsible for the morbidity and mortality caused by pneumococci once they have infected the host. In spite of the vast number of publications on *S. pneumoniae*, many questions about its virulence are still unanswered [6], and this pathogen remains a major causative agent of serious human disease, especially community-acquired pneumonia. Although improved vaccines against *S. pneumoniae* are likely to be developed in the near future, a better understanding of the virulence factors determining its pathogenicity will be needed to cope with the devastating effects of pneumococcal disease in humans. The present investigation focus on the structural and functional analysis of pneumolysin protein involved in pore forming in the host cell membranes and inducing inflammatory events.

[II] MATERIALS AND METHODS

2.1 Retrieval of pneumolysin sequence

Pneumolysin protein sequence was retrieved from UniprotKB database [10]. UniprotKB is the central hub for the collection of functional information of proteins.

2.2 Primary structure prediction

The primary structure was predicted using ProtParam tool [1]. For physio-chemical characterization, theoretical isoelectric point (pI), molecular weight, total number of positive and negative residues, extinction coefficient [2], instability index [3] aliphatic index and grand average hydropathy (GRAVY) [7] were computed using the Expasy's). Protparam server (http://expasy.org/cgi-bin/protpraram)

2.3 Secondary structure prediction

The secondary structure was predicted using FASTA sequence of pneumolysin by SOPMA according to the method of Geourjon and Deleage (2006) [7]. It was employed for calculating the seconbil.ibcp.fr/cgi-bin/npsa_autodary structural features of the selected protein sequence. http://npsa-pmat.pl ?page=/NPSA/npsa_sopma.html

2.4 Protein functional sites

InterPro scan and Fingerprint scan are the tools used to predict the signatures and the motif regions in the sequence.

2.5 Homology Modeling

The protein sequence was subjected for comparative homology modeling via Swiss model according to the method of Arnold [8] to generate putative 3D model. SWISS-MODEL is fully automated protein structure homology modeling server to make the protein modeling accessible to all biotechnologist. The SWISS MODEL performs the sequence alignments and searches for the putative template protein for generating the 3D model.

2.6 Structure validation using PROCHECK

According to the method of Laskowski [9]. PROCHECK checks the stereochemical quality of a protein structure, producing a number of PostScript plots analyzing its overall residue-by-residue geometry. It includes PROCHECK-NMR for checking the quality of structures solved by NMR. The structure was visualized and analyzed in RasMol.

[III] RESULT

3.1 Protein primary sequence analysis

The primary structure of pneumolysin was predicted using Expasy's ProtParam server (http://expasy.org/cgi-bin/protparam). The result revealed that pneumolysin had 471 amino acid residues and molecular weight 52898.4. The maximum number of amino acids present in the sequence was found to be Valine (9.6%) and least was that of Cysteine (0.2%).The total number of negatively charged residues (Asp+Glu) was 65 and the total number of positively charged residues (Arg+Lys) was 51.The isoelectric point pI was 5.18, protein is acidic in nature. The high aliphatic index (86.86).While instability index 20.69.The ground average hydropathicity (GRAVY) is very low -0.420.

3.2 Secondary structure prediction of pneumolysin

The secondary structure is composed of alpha helix and beta sheets and is predicted by SOPMA as shown in Table-1.The secondary structure prediction was done and random coil was found to be 40.98% followed by extended strand 26.33% **[Figure-1].**

The protein 3D structure was built using SWISS-MODEL and the template target alignment was done **[Figure-2].**

The highest identity template was m332 the model quality was checked using PROCHECK. The model quality was obtained about 87.5. % as shown in **[Figure-3].**

The domain analysis was done using Pfam and functional domains were obtained shown in **[Figure-4].**

3.3 Protein functional sites

Interproscan and Fingerprint scan were the tools used to predict the signatures and the motif regions in the protein as shown in the Table-2.

[IV] CONCLUSION

This approach enables rapid potential drug target identification, thereby greatly facilitating the search for new antibiotics. Novel inhibitors can be designed against pneumolysin. This *In Silico* approaches reduces the effort of wet lab and also increases the probability of success. By this present study we have tried to evaluate the target could be better target for rational drug designing.

[V] REFERENCE:

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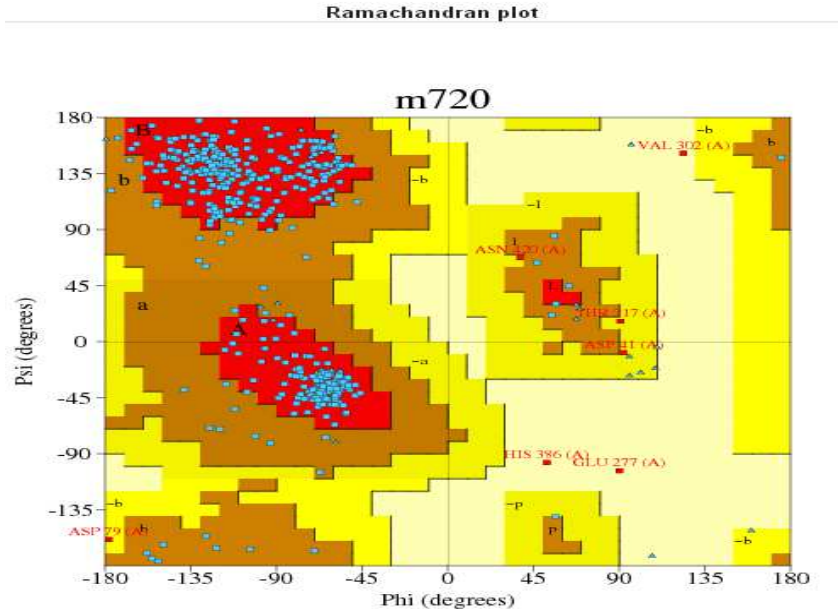
Secondary structure	SOPMA
Alpha Helix	23.78%
3_{10} Helix	0.00%
Pi Helix	0.00%
Beta Bridge	0.00%
Extended Strand	26.33%
Beta Turn	8.92%
Bend Region	0.00%
Random Coil	40.98%
Ambiguous States	0.00%
Other States	0.00%

Table: 1. Secondary structure prediction of pneumolysin by SOPMA

Fig: 2. Three dimensional structure of pneumolysin.



		No. of residues	%-tage
Most favoured regions	[A,B,L]	371	87.5%*
Additional allowed regions	[a,b,l,p]	46	10.8%
Generously allowed regions	[~a,~b,~l,~p]	4	0.9%
Disallowed regions	[XX]	3	0.7%*
Non-glycine and non-proline residues		424	100.0%
End-residues (excl. Gly and Pro)		2	
Glycine residues		26	
Proline residues		17	
Total number of residues		469	

Fig: 3. Ramachandran plot of pneumolysin.

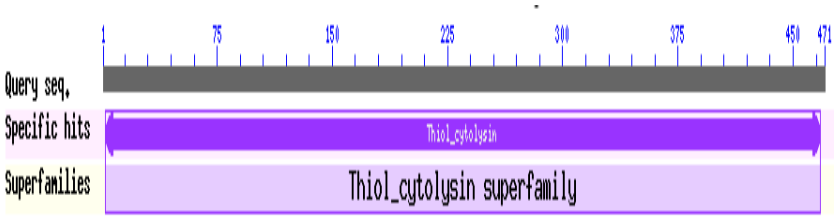


Fig:4. Conserved domain analysis of pneumolysin by pfam.

Finger PRINT	No. of motifs
TACYTOLYSIN	11
LEXASERPTASE	2
BETATUBULIN	2
LVDCCANPHAIC	2
TBOX	2
AQUAPORIN6	2
GGTRANSPTASE	2
PEROPSIN	2
PHEROMONEBAR	2
HEATSHOCK70	2

Table: 2. Fingerprints scan result of pneumolysin protein.