

Evaluation of ZnuA (Zinc-Binding Protein) Linear and Conformational Epitopes in *Propionibacterium acnes*

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Abstract: *Actinobacillus pleuropneumoniae* is the causative agent of acute and chronic pleuropneumonia that is responsible for substantial morbidity and mortality in the pig industry. New improved vaccines that can protect against all serotypes and prevent colonization are required. Previous studies showed that whole cells of *Propionibacterium acnes* protected pigs from *A. pleuropneumoniae* serotype 1 and 5 and therefore the basis for a promising heterologous vaccine. The aim of this study was to identify those protein antigens of *P. acnes* responsible for protection against *A. pleuropneumoniae* infection. Antibodies that are induced by recombinant PASsb cross-react with epitopes in the ApxIV and ZnuA proteins of *A. pleuropneumoniae*. ZnuA is a known *A. pleuropneumoniae* immunogenic protein and is required for virulence.

Key words: *Propionibacterium*, ZnuA, linear and conformational epitopes, bioinformatic, Iran

INTRODUCTION

Acute and chronic respiratory infection caused by *Actinobacillus pleuropneumoniae* is responsible for substantial losses in the worldwide pig industry (Pattison *et al.*, 1957). Vaccines are the mainstay of control but typically only protect against strains of the same serotype and do not prevent carriage. Researchers has been evaluating the potential of heterologous vaccines for *A. pleuropneumoniae* based on *Propionibacterium acnes* (Ramjeet *et al.*, 2008). Whole cells of *P. acnes* strain S4 protected pigs and mice from infection by *A. pleuropneumoniae* serotypes 1 and 5 (Lei *et al.*, 2008). About 2-DE and immunoblotting experiments identified six cross-reactive *P. acnes* proteins including single strand DNA-binding protein. Similarity between small peptide sequences present in *P. acnes* single strand DNA binding protein and *A. pleuropneumoniae* ApxIV toxin and zinc transporter protein ZnuA may contribute to the cross-protection observed.

Antibodies that are induced by recombinant PASsb cross-react with epitopes in the ApxIV and ZnuA proteins of *A. pleuropneumoniae* (Dowell *et al.*, 2005). *P. acnes* induced immunity to *A. pleuropneumoniae* serotype 1 and 5 may be mediated by PA-Ssb eliciting cross-reactive antibodies to ApxIV and ZnuA (Mansfield, 1995). There is high homology between peptides present in PA-Ssb and ApxIV and ZnuA in many *A. pleuropneumoniae*

serotypes. This suggests that *P. acnes* may protect against *A. pleuropneumoniae* serotypes other than 1 and 5 and will be the subject of further evaluation. Six *P. acnes* protein antigens that were recognized by sera raised against *A. pleuropneumoniae* were identified by 2-DE and immunoblotting. Recombinant versions of all *P. acnes* proteins gave partial protection (10-80%) against *A. pleuropneumoniae* serotype 1 and/or 5 infection in a mouse challenge model. The best protection (80% serotype 1; 60% serotype 5) was obtained using recombinant *P. acnes* single-stranded DNA-binding protein. In part, protection against A (Bruggemann *et al.*, 2004). Pleuropneumoniae infection may be mediated by small peptide sequences present in *P. acnes* single-stranded DNA-binding protein that are cross-reactive with those present in the *A. pleuropneumoniae* specific RTX toxin ApxIV and the zinc-binding protein ZnuA. The results suggest that *P. acnes* may be a useful vaccine to protect against different serotypes of *A. pleuropneumoniae* (Shibusawa *et al.*, 2003). ZnuA is a known *A. pleuropneumoniae* immunogenic protein (Schaller *et al.*, 1999) and is required for virulence (Turni and Blackall, 2007; Buettner *et al.*, 2011).

Now a days, bioinformatic tools are of interesting advantages for biologists. Prediction of 3D protein structure is one of the wide applications of these tools (Kafee and Sefid, 2016; Payandeh *et al.*, 2015). Several methods and algorithms are available for protein structure predictions, homology modeling being one of

them. Homology modeling is an in silico method for prediction of 3D protein structures based on known homologous protein structures as a template (Farahmandian *et al.*, 2016). New genome analysis tools based on bioinformatics and immunoinformatics approaches help us select suitable antigens or epitopes directly from the genomes of pathogens in order to design a vaccine. These tools could be employed for epitope selection and vaccine design. Moreover, prediction of protein structures is one of their wide applications (Asefeh *et al.*, 2016; Bastani and Sefid, 2016; Kofeiti *et al.*, 2016; Dehghani and Fateme, 2016; Sefid *et al.*, 2013, 2015, 2016; Darbandian and Sefid, 2017).

The aim of this study was to identify linear and conformational epitopes of znuA protein in *Propionibacterium acnes* and determine its vaccine potential to protect against propionibacterium challenge.

MATERIALS AND METHODS

Sequence availability: ZnuA protein sequence with accession No. GAE67435.1 obtained from NCBI at <http://www.ncbi.nlm.nih.gov/protein> was saved in FASTA format for further analyses.

3D modeling: Phyre2 at <http://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id=index> uses the alignment of hidden Markov models via HHsearch to significantly improve accuracy of alignment and detection rate. Phyre2 also incorporates a new ab initio folding simulation called Poing to model regions of proteins with no detectable homology to known structures.

Phyre2 is a major update to the original phyre server. A range of new features have been included, accuracy has been substantially improved and the interface has been redesigned to be more intuitive and powerful. Poing is also used to combine multiple templates. Distance constraints from individual models are treated as linear elastic springs. Poing then synthesises your entire protein in the presence of these springs and at the same time models unconstrained regions using its physics simulation.

Single-scale amino acid properties assay: Segments within ZnuA sequence that are likely to be antigenic were predicted using bcepred (Sefid *et al.*, 2015) at <http://www.imtech.res.in/raghava/bcepred> with accuracy of 58.7%. This server predicts B-cell epitopes using single of the physico-chemical properties (hydrophilicity, flexibility, mobility, accessibility, polarity, exposed surface and turns) or combination of them. parameters such as hydrophilicity, flexibility, accessibility, turns and antigenic

propensity of polypeptide have been correlated with the location of B-cell epitopes. This has led to a search for empirical rules that would allow the position of B-cell epitopes to be predicted from certain features of the protein sequence.

Sequence-based B-cell epitope prediction: All servers mentioned in this section were used to ascertain B-cell epitopes in ZnuA. ABCpred at <http://www.imtech.res.in/raghava/abcpred/predicts> B-cell epitope(s) in an antigen sequence, using artificial neural network. This is the first server developed based on recurrent neural network (machine based technique) using fixed length patterns. Window length used for prediction with 0.85 threshold set as 16-mer and overlapping filter was on. BepiPred at <http://www.cbs.dtu.dk/services/BepiPred/> predicts the location of linear B-cell epitopes using a combination of a hidden Markov model and a propensity scale method.

Structure-based B-cell epitope prediction: ZnuA 3D structure served as an input file for servers predicting B-cell epitopes based on 3D structure of a given protein. In the following of the study the servers employed will be detailed. EPCES at <http://sysbio.unl.edu/EPCES/> Predicts antigenic epitopes on protein surfaces. EPCES uses consensus scoring (EPCES) from six different scoring functions-residue epitope propensity, conservation score, side-chain energy score, contact number, surface planarity score and secondary structure composition. ElliPro at <http://tools.immuneepitope.org/tools/ElliPro/tutorial.jsp> predicts linear and discontinuous antibody epitopes.

RESULTS AND DISCUSSION

Sequence availability: The protein sequence with 224 residue obtained from NCBI and saved in FASTA format. Protein sequence serving as query for BLAST produced a set of sequences as the highest similar sequence.

3D structure prediction: Building a model comprises four main steps: identification of structural template(s), alignment of target sequence and template structure(s), model building and model quality evaluation. These steps can be repeated until a satisfying modelling result is achieved. Each of the four steps requires specialized software and access to up-to-date protein sequence and structure databases. The 153 residues (68% of sequence) have been modelled with 100.0% confidence by the single highest scoring template. Phyre2 3D structure prediction is shown in Fig. 1.

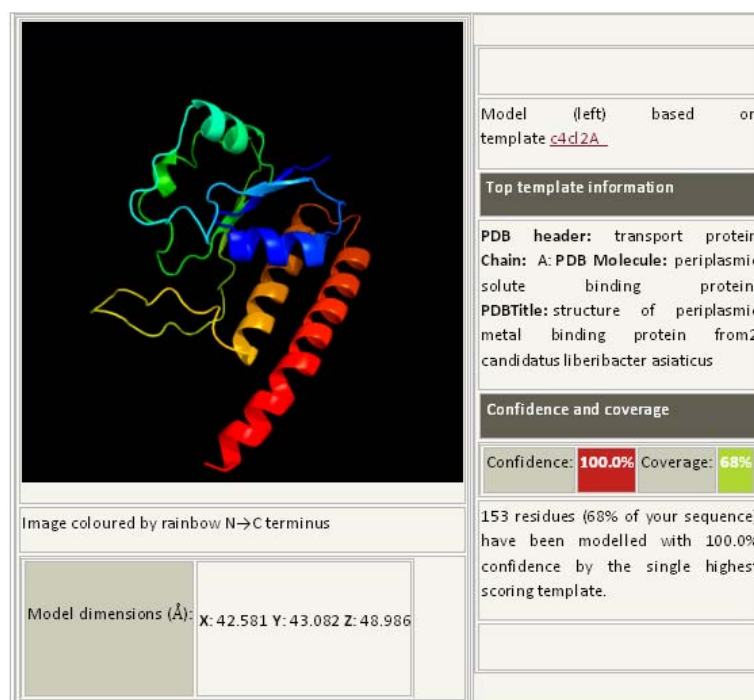


Fig. 1: Phyre2 3D structure prediction

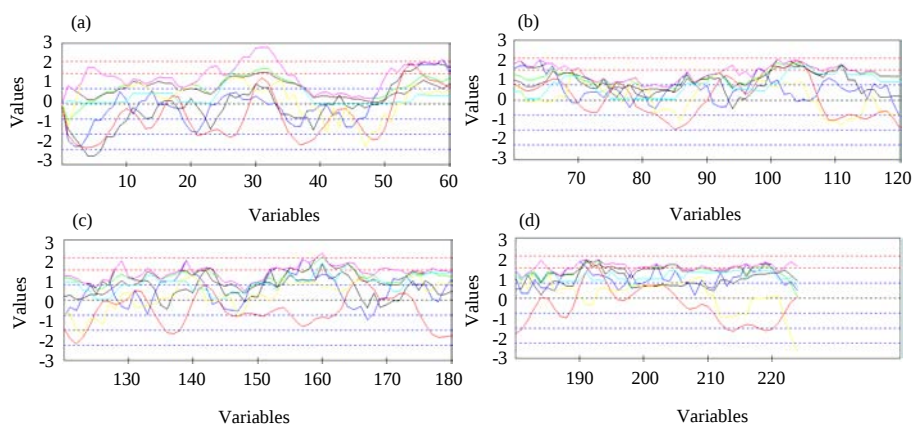


Fig. 2: Bcepred hydrophilicity, accessibility, antigenicity, flexibility and beta turn secondary structure prediction in the protein sequence

Single-scale amino acid properties assay: Propensity scale methods assign a propensity value to each amino acid which measures the tendency of an amino acid to be part of a B-cell epitope (as compared to the background). To reduce fluctuations, the score for each target amino acid residue in a query sequence is computed as the average of the propensity values of the amino acids in a sliding window centered at the target residue.

Hydrophilicity, accessibility, antigenicity, flexibility and secondary structure properties have fundamental role in B-cell epitope prediction. Relying on just one of these

properties, reliable results could not be achieved. Antigenicity plot displays the variation of the antigenic index as function of amino acid position. The higher the antigenic index the more likely should be that antibodies would "see" those groups of residues. Thus, we combined all the data obtained from various servers and software to predict the B-cell epitopes.

Bcepred server predict several properties such as hydrophilicity, accessibility, antigenicity, flexibility and beta turn secondary structure in the protein sequence (Fig. 2). Although, single-scale amino acid properties were

Table 1: Linear B-cell epitopes predicted by ABC pred server

Ranks	Sequence	Start position	Score
1	LLLMFSLPMRFMNTT	4	0.92
2	VHSINSPDQDPHDYE	93	0.91
3	AGCSNSGPDSSGSPSA	50	0.87
3	AGFIDAVETSLKKPG	145	0.87
4	GSVAQAIGGDKVNVHS	80	0.86
5	PSASSDKVDVVASTNV	63	0.83
5	PDSGSGPSASSDKVDV	57	0.83
5	KIAIANGGGYDDWATK	121	0.83
6	KVVESDLVKASPDNKT	180	0.81
7	VFYSIDSVRKVAKVVE	168	0.80
7	GGYDDWATKLIKSTSP	128	0.80
8	ACALAMAGCSNSGPDSS	44	0.79
9	TDLKARATKVGKAST	209	0.77
9	TMVITYVLEREHHMHR	19	0.77
10	VDVVASTNVWGSVAQA	70	0.76
10	KASPDNKTTFETNLKN	188	0.76
11	KLIKSTSPQAGFIDAV	136	0.70
12	SPDQDPHDYEATAKDK	99	0.66
13	SGLKKPGQKEFNEHVF	154	0.65
14	TFETNLKNFESKLT	195	0.64
14	LMRFMNTTMVITYVL	11	0.64
14	DYEATAKDKLAFSKAK	106	0.64
15	VLEREHHMHRLLAIA	25	0.61

Table 2: Linear epitopes predicted by Ellipro

Start	End	Peptide	No. of residues	Score
206	222	SKLTDLKARATKVGKKA	17	0.808
154	166	SGLKKPGQKEFNE	13	0.725
98	115	NSPDQDPHDYEATAKDKL	18	0.685
136	145	KLIKSTSPQA	10	0.664
588	91	GDKV	4	0.575
131	134	DDWA	4	0.515

detectable in all sequence length, peaks in the plot indicate putative susceptible epitope boundaries. Termini hits to the first and last 10 residues of the protein sequence were not remarkable these properties points of view.

Prediction of B-cell epitopes by integrated strategy:

B-cell epitopes are regions on the surface of antigens that are recognized by B-cell receptors or specific antibodies. These epitopes can be categorized into two types: linear (continuous) and conformational (discontinuous) epitopes. Linear epitopes comprise continuous residues in a sequence while conformational epitopes consist of distantly separated residues in the sequence so that spatially adjacent. Linear and conformational epitopes in znuA protein predict by several softwares and various algorithms. Linear B-cell epitops predicted by bepiped server are shown in Fig. 3. ABCpred result shows 15 hits of 16 meric peptide sequences as B-cell epitopes ranking based on scores (Table 1). The predicted B-cell epitopes are ranked according to their score obtained by trained recurrent neural network. Higher score of the peptide means the higher probability to be as epitope. All the peptides shown here are above the threshold value chosen.

Linear and discontinuous B-cell epitopes were predicted by ElliPro Software are shown in Table 2 and 3. In Fig. 4, two discontinuous and 2 linear

Table 3: Discontinuous epitopes predicted by Ellipro

Residues	No. of residues	Score
_:K69, _:V70, _:G88, _:D89, _:K90, _:V91, _:V187, _:K188, _:A189, _:S190, _:P191, _:D192, _:N193, _:K194, _:T195, _:T196, _:E198, _:T199, _:K202, _:N203, _:S206, _:T209, _:D210, _:L211, _:K212, _:A213, _:R214, _:A215, _:T216, _:K217, _:V218, _:G219, _:K220, _:K221, _:A222	35	0.747
_:Y107, _:E108, _:A109, _:T110, _:A111, _:K112, _:D113, _:K114, _:A134, _:K136, _:L137, _:I138, _:K139, _:S140, _:T141, _:S142, _:P143, _:Q144, _:A145	19	0.687
_:G128, _:S154, _:G155, _:L156, _:K157, _:K158, _:P159, _:G160, _:Q161, _:K162, _:E163, _:F164, _:N165, _:E166, _:H167	15	0.672
_:N98, _:S99, _:P100, _:D101, _:Q102, _:D103, _:P104, _:H105, _:D106, _:G129, _:D131, _:D132, _:W133	13	0.585
_:L115, _:S118, _:K119, _:A120	4	0.565

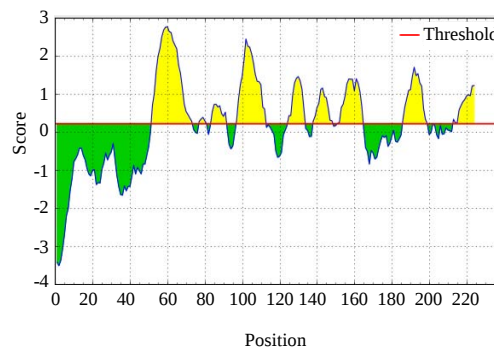


Fig. 3: Linear B-cell epitops predicted by bepiped server

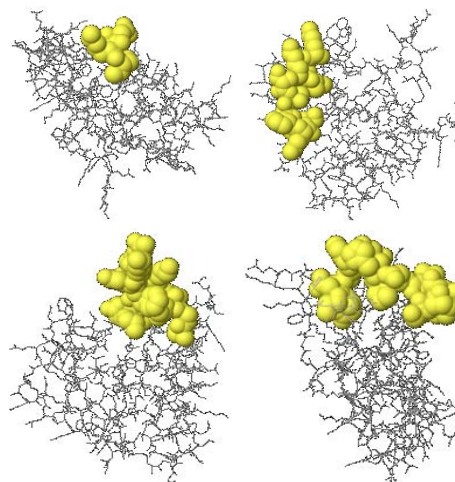


Fig. 4: About 2 linear (up) and 2 discontinuous (down) epitopes with the highest PI score predicted by ellipro server are shown. Epitopes mapped on 3D models using Discovery Studio Visualizer 2.5.5 Software

epitopes with the highest PI (protrusion index). "SKLTDLKARATKVGKKA" at position 206-222 and "SGLKKPGQKEFNE" at position 154-166 is the best linear epitope determined by Ellipro.

CONCLUSION

The aim of this study was to identify linear and conformational epitopes of znuA protein in *Propionibacterium acnes* and determine its vaccine potential to protect against propionibacterium challenge.

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