Research Article

Prediction of Peptide Vaccine Candidate for Dairy Products Borne Human Botulism

Pallavi Mishra, S.Velavan
Department of Life sciences, VELS University, Pallavaram, Chennai, Tamil Nadu, India.
Department of Biochemistry, Marudupandiyar college Thanjavur, Tamil Nadu, India.
***Corresponding author's E-mail:** menagamuniraj@yahoo.com

Accepted on: 24-06-2013; **Finalized on:** 31-07-2013.

ABSTRACT

The raw milk and other dairy products are sometimes contaminated by human botulism causing pathogen, *Clostridium botulinum*, due to insufficient thermal processing and post-process contamination during milk pasteurization treatment. As a result toxic substances like neurotoxin A, B, E, and F are secreted causing human botulism which causes serious illness and paralysis of muscles. The treatment for botulism is only through the application of immunogenic antitoxins. Available antigens are not so specific and effective. The present investigation applies bioinformatics tools by which specific and effective peptide vaccine for dairy products borne human botulism has been proposed.

Keywords: Bioinformatics, *Clostridium botulinum*, Human botulism, Neurotoxins, Peptide vaccine.

INTRODUCTION

Human botulism risk is associated with the consumption of dairy products. Multiplication of Clostridium botulinum causes contamination of the raw milk and further transmission through the dairy chain are possible. The standard milk pasteurization treatment does not eliminate spores, and allow botulinal growth and toxin production. Several large botulism outbreaks due to both commercial and home-prepared dairy products have been reported. Factors explaining these outbreaks, not only include temperature abuse, but also unsafe formulation, inadequate fermentation, insufficient thermal processing and post-process contamination. The small number of outbreaks is probably explained by a low incidence of spores in milk, the presence of competitive bacteria in pasteurized milk and other dairy products, and growth-inhibitory combinations of intrinsic and extrinsic factors in cultured and processed dairy products. There are four strains of botulism; types A, B, E, and F cause human botulism each producing a potent protein neurotoxin. Botulism causes serious illness and paralysis of muscles which is caused by a neurotoxin, produced by the bacterium *Clostridium botulinum.*[1]

The only drug currently available to treat infant botulism is Botulism Immune Globulin Intravenous-Human (BIG-IV or Baby BIG). If diagnosed early, foodborne and wound botulism can be treated by inducing passive immunity, which blocks the action of toxin circulating in the blood. There are two primary botulinum antitoxins available for treatment of wound and foodborne botulism. They are trivalent (A,B,E) and heptavalent (A,B,C,D,E,F,G) botulinum antitoxin which is derived from IgG antibodies which are less immunogenic antitoxins.[2] Therefore it is essential to identify effective highly immunogenic peptide based antigen. In the current work it has been proposed to analyze the neurotoxins of *C. botulinum* using Bioinformatics tools and to produce effective highly immunogenic peptide antigen against botulism.

MATERIALS AND METHODS

Amino acids sequences of Botulism Neurotoxin (BoNT) A,B,E and F of *C. botulinum* were derived from Protein data bank.[3] The protein antigens were tested for their antigenic property using VaxiJen[4], TMHMM.[5] The enzyme having highest antigenicity score was selected for the prediction of B-cell epitopes using ABCPreds.[6] B-cell eiptopes having highest score were selected for the prediction of T-cell epitopes using Propred-1[7], Propred.[8] The population study was carried out using MHCPred[9] and T-epitope Designer.[10] Peptide Design Tool [11] was used to confirm the suitability of predicted T-cell epitopes for antibody production.

RESULTS AND DISCUSSION

In human beings, botulism is caused by *C. botulinum* by secreting neurotoxin type A,B,E and F. These are enzymes which can immunologically act as antigens which induce antitoxins in humans as antibodies. All enzymes are not antigens. Therefore it is essential to identify all these types A, B, E and F neurotoxins to test for their antigenicity. An antigen must have antigenicity score more than 0.4 and it should be exomembrane in topology.[12] In this study VaxiJen server for antigenicity score and TMHMM tool for exomembrane topology prediction were used. Based on the results, neurotoxin types B, E and F were identified as antigens because of their exo-membrane topology and VaxiJen score more than the threshold value 0.4 (Table 1). Neurotoxin type A was non antigen due to the VaxiJen score less than the threshold value of 0.4, in spite of its exomembrane topology.

Among the three types of neurotoxins, type E which showed highest VaxiJen score was selected for the

peptide vaccine selection. In this process, the amino acid sequence of the neurotoxins type E was subjected to B-cell epitope prediction. Using the bioinformatics tool ABCPreds [6], number of different types of B-cell epitopes each having 16-mer were predicted. Among the B-cell epitopes, the predicted B cell epitopes were ranked according to their score obtained by trained recurrent neural network (Table 2). High score of the peptide means the higher probability to be as epitope. Among the predicted B-cell epitopes, two epitopes HNEIIWTLQDNAGINQ and YIGIRYFNIFDKELDE with highest scores were selected for analysis. The B-cell epitope, HNEIIWTLQDNAGINQ inspite of positive antigenicity score (Figure 1) was not selected due to the absence of exomembrane topology (Figure 2). On the otherhand YIGIRYFNIFDKELDE was selected due to the antigenicity score more than 0.4 (Figure 3) and exomembrane topology (Figure 4).

Table 1: Antigenicity of neurotoxins causing human botulism

Names of neurotoxin (enzymes)	VaxiJen score	Topology	Antigenicity
Neurotoxin type-A	0.3889	Exomembrane	Non-Antigen
Neurotoxin type-B	0.4109	Exomembrane	Antigen
Neurotoxin type-E	0.5246	Exomembrane	Antigen
Neurotoxin type-F	0.5040	Exomembrane	Antigen

Table 2: List of predicted B-cell epitopes from neurotoxin E

Predicted B-cell epitope

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.

Rank	Sequence	Start position	Score
1	HNEIIWTLQDNAGINQ	956	0.97
1	YIGIRYFNIFDKELDE	1041	0.97
2	PKEIDDTVTSNNNYEN	451	0.96
3	GGCQEFYKSFNIMKNI	24	0.95
3	AKGITTKYTITQKQNP	224	0.95
4	PNVIIMGAEPDLFETN	146	0.93
5	FVTITNDRLGDSKLYI	992	0.92
5	SSVLNMRYKNDKYVDT	850	0.92
5	NPRIITPITGRGLVKK	392	0.92
6	EVKINKLREYDENVKT	776	0.91
6	FNSESAPGLSDEKLNL	475	0.91
6	DDNINTPKEIDDTVTS	445	0.91
7	SFWVRIPNYDNKIVNV	918	0.90
7	TSSIDTALLEQPKIYT	538	0.90
7	MNSVGNNCTMNFKNNN	1190	0.90
8	YKKIASKLSKVQVSNP	279	0.89
8	VASTWYYTHMRDHTNS	1220	0.89
8	ETEIQTLYSNEPNTNI	1056	0.89

The selected B-cell epitope was subjected to T-cell epitope prediction. The predicted T-cell epitopes should bind with atleast 15 MHC alleles of class I and II and also should bind with common HLA alleles A*0201, A*0204, B*2705, DRB1*0101and DRB1*0401 of the human population with high positive score.[13] In this study the selected B-cell epitope predicted three kinds of T-cell epitopes out of which only one qualified among them, by binding with 16 MHC-I and II alleles(Figure 5 and 6) and having 99% score with human HLA alleles (Table 3). The selected T-cell epitope also showed a high antigenicity score of 0.8727 which is more than 0.4 (Figure 7). An ideal antigen that can produce both B-cell and T-cell mediated immunity is highly useful. In the present study, since the T-cell epitopes were predicted from B-cell epitopes, could produce both B-cell and T-cell mediated immunity.

This T-cell epitope is now eligible to act as a third generation peptide antigen which expected to be as a highly immunogenic antigen which will induce the production of antibodies to overcome the dreadful disease human botulism. To confirm the eligibility of this antigen to act as an ideal vaccine, this peptide vaccine candidate WTLQDNAGI was submitted to peptide station, innovagen at Sweden (Figure 8).[11] They have reported that this peptide is suitable for antibody production against antigen causing human botulism.

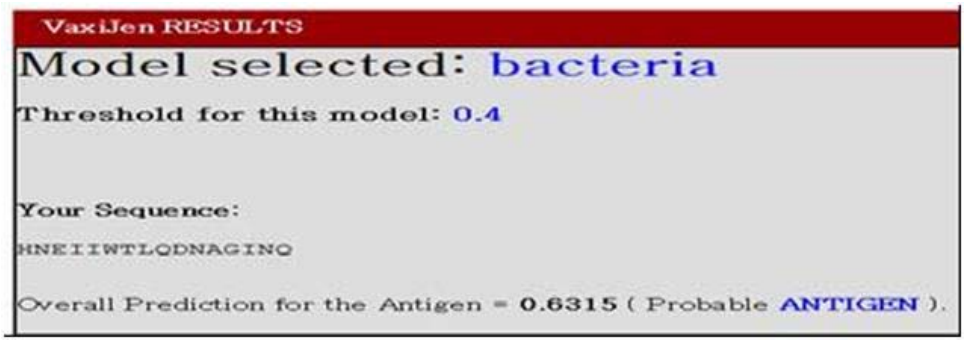

Figure 1: Prediction of antigenicity of B-cell epitope HNEIIWTLQDNAGINQ (Note the antigenic nature of B-cell epitope)

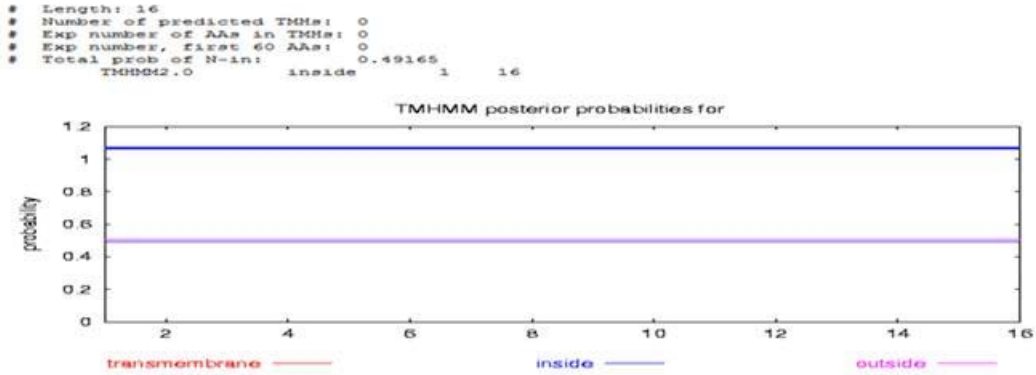

Figure 2: Exomembrane topology of B-cell epitope HNEIIWTLQDNAGINQ (Note the absence of exomembrane topology)

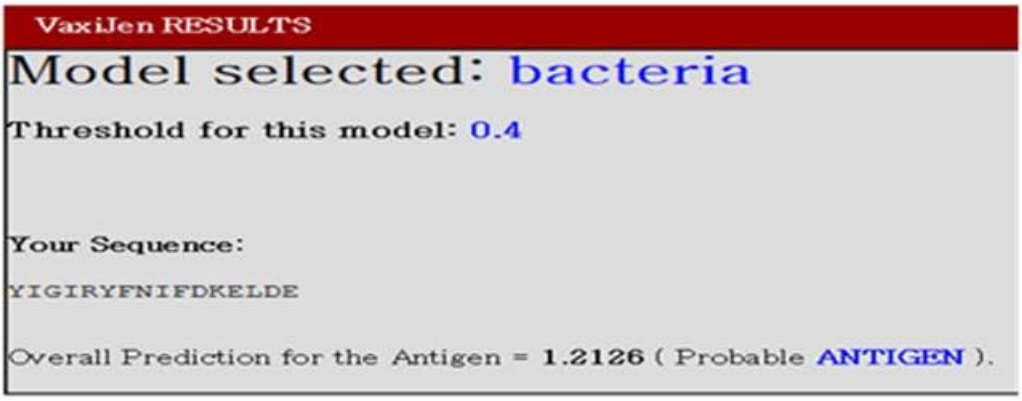

Figure 3: Prediction of antigenicity of B-cell epitope YIGIRYFNIFDKELDE (Note the antigenic nature of B-cell epitope)

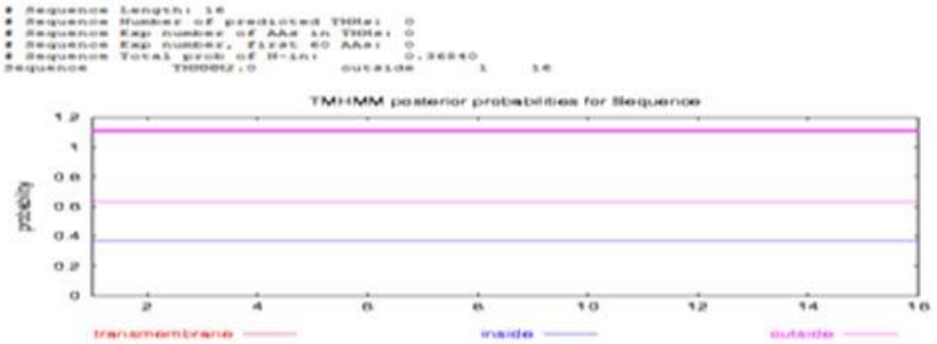

Figure 4: Exomembrane topology of B-cell epitope YIGIRYFNIFDKELDE (Note the exomembrane topology)

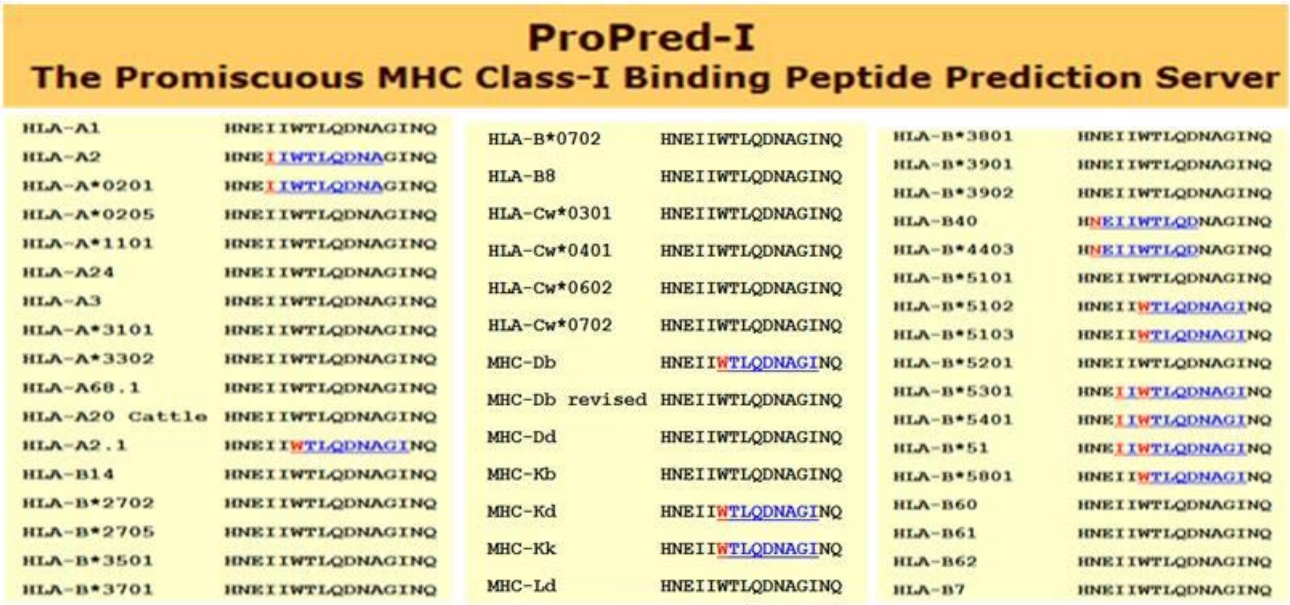

Figure 5: Prediction of MHC-I binding T-cell epitopes from B-cell epitopes

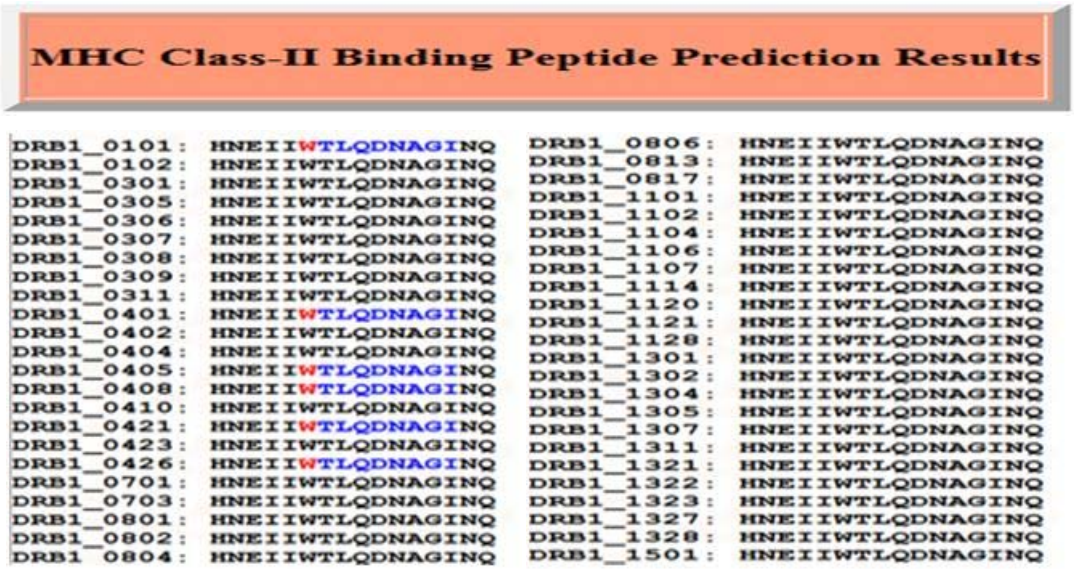

Figure 6: Prediction of MHC-II binding T-cell epitopes from B-cell epitopes

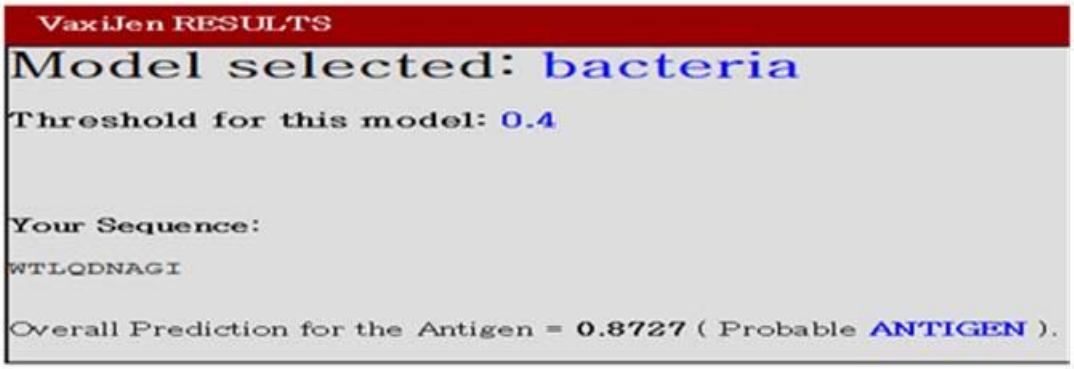

Figure 7: Prediction of antignicity of T-cell epitope, WTLQDNAGI

Table 3: The binding ability of T-cell epitopes with HLA molecules of human population

Antigens (Proteins)	Selected T-epitopes	T-Epitope Designer			MHCPred (IC50 Value)		Highest score of T-cell epitope with HLA alleles of population
		A*0201	A*0204	B*2705	DRB1*0101	DRB1*0401	
BoNT-E	**WTLQDNAGI**	280.08	459.25	783.68	217.27	**285.10**	**99%**

Figure 8: Confirmation of vaccine candidate for the antibody production

CONCLUSION

To overcome the human botulism caused by Neurotoxin (BoNT) A, B, E and F of *C. botulinum* is a major challenge to scientific community. This challenge can be faced successfully either with antibiotics or with immunologic treatment. At present the available treatments are with less immunogenic antibodies because of their antigens which are bread based and prepared from first are second generation antigens. On the other hand, in the present study the proposed peptide antigen is precisely designed using immuno-informatics analysis. Such a third generation antigen definitely will produce effective antibodies to overcome the dreadful disease, human botulism.

Acknowledgement: The authors are thankful to Dr.M.Menakha, Faculty of Microbiology and Bioinformatics INI Centre for Research and Cognizance (ICRAC) for the expertise support in bioinformatics and Dr. K. Karthikeyan INI Centre for Research and Cognizance (ICRAC), Chennai for the help rendered during the discussion.

REFERENCES

1. Lindström M, Myllykoski J, Sivelä S, Korkeala H, *Clostridium botulinum* in cattle and dairy products, Crit Rev Food Sci Nutr, 50(4), 2010, 281-304. PMID: 20301016.
2. Schantz EJ, Johnson EA, Properties and use of botulinum toxin and other microbial neurotoxins in medicine, Microbiol Rev, 56(1), 1992, 80-99.
3. Berman HM, The Protein Data Bank: a historical perspective, Acta Crystallogr A, 64(1), 2008, 88-95. Epub 2007 Dec 21.
4. Doytchinova IA, Flower DR, VaxiJen: a server for prediction of protective antigens, tumour antigens and subunit vaccines, BMC Bioinformatics, 8, 2007, 4.
5. Krogh A, Larsson B, von Heijne G, Sonnhammer EL,. Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes, J Mol Biol, 19, 305(3), 2001, 567-80.
6. Saha S, Raghava GPS, Prediction of Continuous B-cell Epitopes in an Antigen Using Recurrent Neural Network, Proteins, 65(1), 2006, 40-48. PMID: 16894596.
7. Singh H, Raghava GP, ProPred I: prediction of promiscuous MHC class –I binding sites, Bioinformatics, 19(8), 2003, 1009-14.
8. Singh H, Raghava GP, ProPred: prediction of HLA-DR binding sites, Bioinformatics, 17(12), 2001, 1236-7.
9. Guan P, Doytchinova IA, Zygouri C, Flower DR,. MHCPred: bringing a quantitative dimension to the online prediction of MHC binding, *Appl Bioinformatics*, 2, 2003, 63-66.
10. Pandjassarame Kangueane, Meena Kishore Sakharkar, T-Epitope Designer: A HLA-peptide binding prediction server, Bioinformation, 1(1), 2005, 21–24. PMCID: PMC1891623.
11. http://www.innovagen.com/contact.asp
12. Barh D, Misra AN, Kumar A, In Silico Identification of Dual Ability of N. gonorrhoeae ddl for developing drug and vaccine against pathogenic Neisseria and other human pathogens, J Proteomics Bioinform, 3, 2010, 082-090.
13. Barh D, Misra AN, *In silico* Identification of membrane associated candidate drug targets in *Neisseria gonorrhoeae*, International Journal of Integrative Biology, 6, 2009, 65-67.

Source of Support: Nil, **Conflict of Interest:** None.