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In silico epitope analysis of *Shigella dysenteriae* for the design of immunogens in food contaminant detection

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Abstract

Shigella dysenteriae is an intracellular bacterium responsible for shigellosis, a disease prevalent in developing countries with a reported mortality rate of 10–15%. Transmission typically occurs through contaminated food. In addition to maintaining hygiene during food handling and preparation, verifying that food ingredients are free from S. dysenteriae is critical for preventing its spread. Immunoassay-based methods are commonly used for such authentication and rely on the availability of specific antibodies targeting *S. dysenteriae*. The production of these antibodies requires the identification of specific immunogenic epitopes. In this study, we conducted an in silico analysis to identify a potential epitope of S. dysenteriae using bioinformatics tools. Protein sequence data were obtained from UniProtKB (https://www.uniprot.org/) and analysed with the VaxiJen v2.0 server (https://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html). This analysis revealed a candidate immunogenic epitope with the amino acid sequence LAKLTNNNAQGEITDIIALAY. While these findings are promising, further in vivo validation is necessary to determine whether this epitope can effectively elicit antibody production for use in immunoassay development aimed at detecting S. dysenteriae contamination in food.

Keywords: Bioinformatics; Epitope; in silico; *Shigella dysenteriae*; Vaccine

1. Introduction

Contamination of pathogenic bacteria in food and beverages can cause various diseases such as *typhoid*, diarrhea, as well as food poisoning (1). These diseases will easily attack people who experience a decrease in body resistance due to factors from intrinsic and from extrinsic (2,3). Foodborne diseases or food poisoning caused by contamination by pathogenic bacteria need careful attention, because people with these cases can experience digestive disorders and impaired absorption of nutrients (2).

Salmonella, Escherichia coli, Shigella, and a range of other microorganisms are recognized as major contributors to foodborne illnesses (4). *Shigella* is a genus of Gram-negative bacteria, closely related to Escherichia coli, that cause diarrheal disease and have remained a significant public health concern globally (5). Furthermore it was reviewed that Shigella is primarily transmitted through ingestion of food or water contaminated with feces, or via direct person-to-person contact (5). An infectious dose as low as 10 to 100 viable cells can cause illness in a healthy individual. Shigellosis is less common in developed countries, where transmission occurs mainly through the fecal–oral route, whereas in developing countries, both fecal–oral transmission and contamination of food and water contribute to its spread (6,7). According to the CDC's 2016 Annual Report on Laboratory-based Enteric Disease Surveillance, 12,597 cases of Shigella infection were reported across all 52 U.S. states. The majority were caused by *Shigella sonnei* (80.5%), followed by *S. flexneri* (12.6%), *S. boydii* (0.2%), and *S. dysenteriae* (0.1%). It has been reported also that *shigellosis* causes more than 200,000 deaths worldwide (8). To date there is no widely accessible vaccine. The disease caused by *Shigella dysenteriae*

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is becoming resistant to various types of antibiotics, while prevention by vaccine mechanisms is still very limited in use (9).

Prevention of the spread of *Shigella dysenteriae* bacteria through food can be done through hygiene quality control when preparing and serving food (10). In addition, authentication that food ingredients are completely free of *Shigella dysenteriae* must be implemented (11). One practical method, which is relatively faster than bacterial culture, is immunoassay-based testing (12). For the development of immunoassay testing tools, the availability of antibodies, both polyclonal and monoclonal, is required. To produce antibodies, an immunogen must be used to immunize experimental animals such as mice, rabbits, or chickens to obtain IgY (13,14). A practical method currently available for preparing immunogens to produce antibodies from experimental animals is through the use of bioinformatics technology (15,16).

In this study, we report the results of our preliminary research utilizing bioinformatics to create an epitope vaccine against *Shigella dysenteriae* bacteria. It is hoped that this vaccine can be used to produce home made antibodies for the development of authentic food safety diagnostics free from *Shigella dysenteriae* in the future

2. Material and methods

This research was conducted in silico, using computer simulations and bioinformatics data analysis based on several studies conducted (17,18).

2.1. Use of software and websites

The protein or peptide sequences of *Shigella dysenteriae* bacteria were drawn from the UniProtKB database site (https://www.uniprot.org) which provides complete information about proteins from various organisms. The peptide sequence of *Shigella dysenteriae* bacteria were filtered and downloaded. Following this, the Immune Epitope Database and Analysis Resource (IEDB, https://www.iedb.org/) was immplied for epitope prediction and analysis (19). Sequence peptides with scores above 0.5 and percentile ranks below 2% were used to clasify a higher affinity for binding to MHC molecules, indicating their potential to elicit a strong immune response. Furtheromere epitopes with affinity values >1,000 were predicted to have potential for peptide vaccine development(19,20).

2.2. Determination of Antigenicity Level of Peptides

The antigenicity level of the peptides was determined using VaxiJen 2.0 software (https://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html). VaxiJen 2.0 is able to predict server-based antigens that allow the determination of antigen potential (21).

2.3. Analysis of Physicochemical Properties

Evaluation of the physicochemical properties of *Shigella dysenteriae* bacterial vaccine candidates was carried out using the Expasy ProtParam online server (https://web.expasy.org/protparam/) (22), and the Pepcalc server (https://pepcalc.com/).

3. Results and Discussion

3.1. *Shigella dysenteriae* protein sequence from the database

Shigella dysenteriae is an intracellular pathogen that causes Shigellosis (bacillary dysentery). Shigellosis is common in developing countries, with a mortality rate of 10-15% if untreated, killing about 1.1 million people out of approximately 120 million cases annually (9). The Gram-negative bacterium *Shigella dysenteriae* type 1 (SD1) is one of the most virulent serotypes of the four Shigella serotypes (*S. Dysentriae, S. Flexneri, S. Sonnei* and *S. boydii*) (23).

In-silico design of peptide vaccine candidates to produce antibodies against *Shigella dysenteriae* bacteria is a research method that utilizes computational technology and databases to develop vaccines without prior laboratory experiments. In this study, the amino acid sequence of *Shigella dysenteriae* bacteria was obtained from UniProtKB (https://www.uniprot.org). It was found as many as 34 thousand types of proteins and gene names from these bacteria, ten of them were chosen in this study (Table 1), and one of them was selected i.e. serotype 1 or *strain Sd 197* with the *entry identifier* number Q329R9, the protein known as *Bifunctional protein GlmU* with456 amino acid length (Figure 1). *Shigella dysenteriae* serotype 1 (SD1), including strain Sd 197, is strongly associated with Shiga toxin (Stx) production (24,25). Stx is the primary virulence factor of SD1 strains and is responsible for the severe diarrheal disease and potential complications like hemolytic uremic syndrome (HUS), reviewed by Wang (25). So, if we succeed in creating

antibodies specific to this strain, it is very possible that we can develop specific diagnostics against Shiga toxin in the food.

Table 1 *Shigella dysentriae* Bacterial Proteins from UniProtKB data

No.	UnitProtKB Identifier	Protein name	Gen name	Organisms	Length
1.	Q9FBI2	Shiga toxin subunit A	stxA	*Shigella dysenteriae*	315 AA
2.	Q7BQ98	Shiga toxin subunit B	stxA	*Shigella dysenteriae*	89 AA
3.	Q32A21	Fatty acid oxidation complex subunit alpha	FadB	*Shigella dysenteriae* serotype 1 (strain Sd197)	729 AA
4.	Q932V0	Alanine recemase. biosynthetic	alr	*Shigella dysenteriae*	359 AA
5.	P00906	Anthranilate synthase component II,	TrpG-TRPD	*Shigella dysenteriae* serotype 1 (strain Sd197)	201 AA
6.	Q326Z6	E3 ubiquitin-protein ligase ipaH9.8,	ipaH9.8	*Shigella dysenteriae* serotype 1 (strain Sd197)	545 AA
7.	Q329R9	Bifunctional protein GlmU	glmU	*Shigella dysenteriae* serotype 1 (strain Sd197)	456 AA
8.	Q32AZ8	Sirome synthase	cysG	*Shigella dysenteriae* serotype 1 (strain Sd197)	457 AA
9.	Q32D45	Dual-specificity RNA Methyltransferase RlmN	rlmN	*Shigella dysenteriae* serotype 1 (strain Sd197)	348 AA
10.	Q32DT3	Bifunctional polymyxin resistance protein ArnA	arnA	*Shigella dysenteriae* serotype 1 (strain Sd197)	660 AA

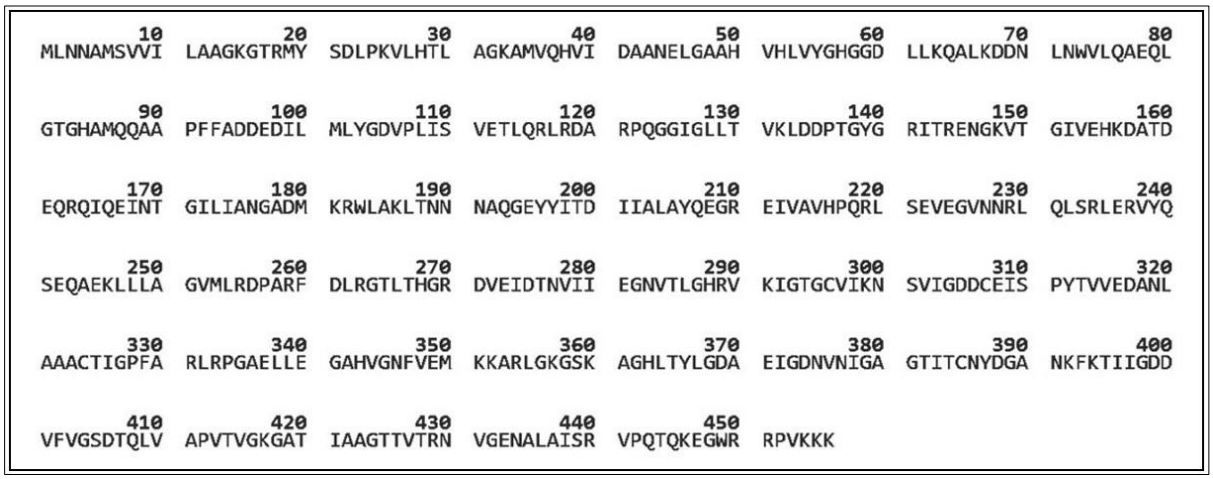

Figure 1 Peptide Sequence of Q32R9 retrieved from UniProtKB

3.2. Determination of peptide antigenicity levels and identification of candidate epitopes

Figure 2 shows the results of sequence alignment analysis using the Immuno-Epitope Database (IEDB). This analysis aims to identify potential epitopes for peptide-based vaccine development (19,20). The distribution and position of epitopes on the graph identified in the SD1 bacterial protein sequence and the colors and symbols on the figure indicate the level of antigenicity and potential of each epitope for vaccine development.

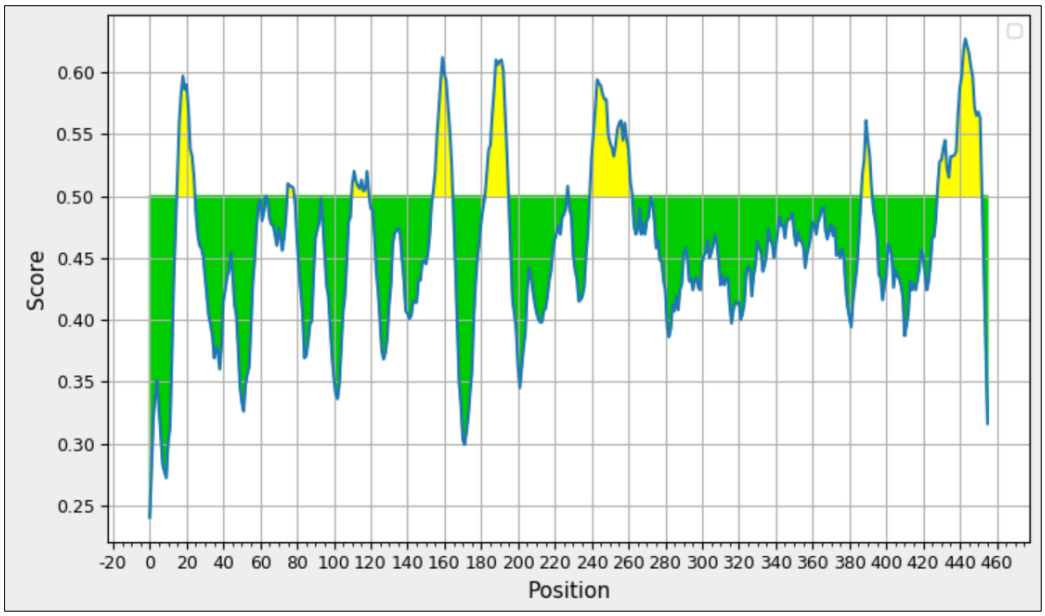

Figure 2 Epitope prediction analysis of *Shigella dysenteriae* bacteria using IEDB

Peptide sequences with antigenic potential were further tested for peptide bond strength, selected based on the results of the IEDB B graph. Table 2, displays the results of this analysis using VaxiJen 2.0 software analysis. Based on the antigenicity score, there is one peptide, LAKLTNNNAQGEITDIIALAY with an antigen binding affinity of 0.5527 Nm (highlighted), which is a potential vaccine candidate for SD1 bacteria.

Table 2 Prediction results of the entigenicity level of *Shigella dysenteriae* bacteria using VaxiJen 2.0 software

Peptide Sequence	**Affinity (Nm)**	**Probabili**
ITDIIALAY	0.1683	Non-antigen
GTRMYSDLPK	-0.0961	Non-antigen
QAEQITDIIALAY	0.1897	Non-antigen
GTRMYSDLPKITDIIALAY	0.1339	Non-antigen
VETLQRLRDAITDIIALAY	-0.1661	Non-antigen
HKDATDEQRQIITDIIALAY	0.3659	Non-antigen
LAKLTNNNAQGEITDIIALAY	0.5527	Antigen
ITDIIALAYN	0.1309	Non-antigen
ITDIIALAYQSEQAEKLLLAGVMLRDPARFDLR	0.3112	Non-antigen
ITDIIALAYDGANKF	-0.1880	Non-antigen
ITDIIALAYRNVGENALAISRVPQTQKEGWRRPV	0.1554	Non-antigen

Peptide-based vaccines have shown potential results in triggering an immune response. However, a single peptide may not be enough to produce a strong and lasting immune response. These vaccines are considered safe as they have fewer side effects. However, it has low immunogenicity when used alone (16,26). Therefore, combining multiple peptides or epitopes in vaccine manufacturing is necessary, with the intention of obtaining a strong immune response resulting in antibodies with sufficient affinity against the pathogen (27).

3.3. Evaluation of Physicochemical properties

Figure 3 displays the results of physicochemical analysis on the *Shigella dysenteriae* bacterial vaccine candidate which has a molecular weight of 2246.52 g/mol, with isoelectric point (pI) occurs at pH 3.93. A pI of 3.93 indicates that the

vaccine is acidic and could lead to instability and poor performance, particularly at the physiological pH of the animal body (around 7.4). In addition, the poor water solubility of this vaccine candidate is also a constraint. When vaccine candidates or drugs have low water solubility, this can lead to several problems, including decreased drug efficacy, irregular or low absorption, and potential side effects or therapeutic inefficacy (28). This can affect the success of the vaccine in stimulating an effective immune response. To overcome these challenges, various strategies are being implemented to increase solubility and improve vaccine efficacy, such as formulation techniques and the use of cyclodextrins (28,29).

Research on the development of a vaccine against *Shigella dysenteriae* using the insilico method is quite advanced, with very promising results, especially for use in humans (8,30,31). In this study, the main objective is that the vaccine is not for use in humans, but for the production of antibodies in experimental animals. The antibodies generated later, will be utilized to develop diagnostic tools for detecting *Shigella dysenteriae* in food. Therefore some limitations found in the study are understandable, given the complexity of this application. However, t whatever the reason, the results this insilico design still needs to be optimized and tested in vitro and in vivo.

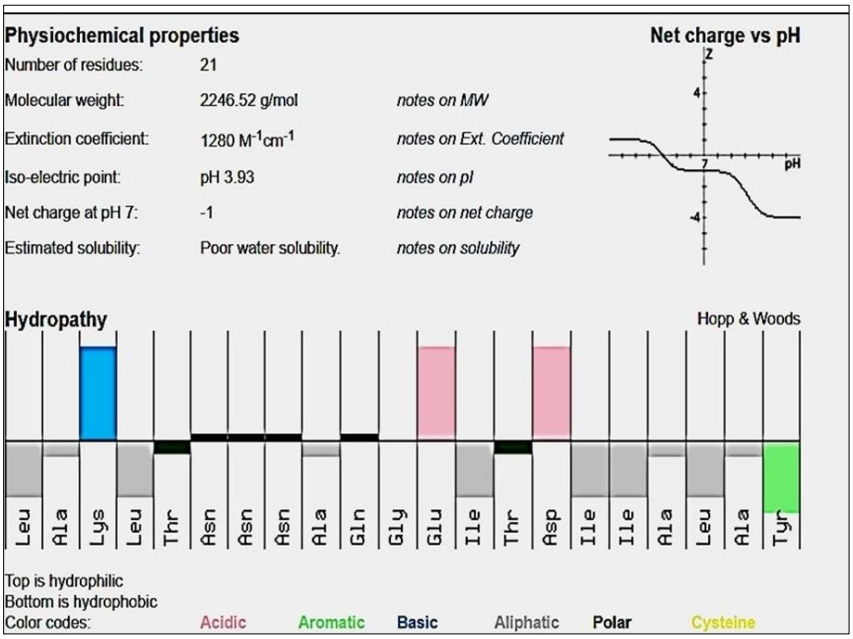

Figure 3 Physicochemical properties analysis using Pepcalc server (https://pepcalc.com/)

4. Conclusion

This study successfully analysis the immunogenic epitopes of *Shigella dysenteriae* as potential candidate epitopes for the development of *Shigella dysenteriae* bacterial vaccines, espesially for the purpose of in-house antibody production. The epitope prediction of *Shigella dysenteriae* bacteria obtained from UniProtKB is LAKLTNNNAQGEITDIIALAY. Further study need to be carried out to ensure whether this epitope is truly effective *in vivo*.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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