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SELECTION OF MIMOTOPE PEPTIDES OF IMMUNODOMINANT EPITOPES OF S. TYPHI LPS USING PHAGE DISPLAY

Armando Navarro-Ocaña
Departamento de Salud Pública Facultad de Medicina Universidad Nacional Autónoma de México, México. Orcid: 0000-0002-6693-7168

Delia Licona-Moreno
Departamento de Salud Pública Facultad de Medicina Universidad Nacional Autónoma de México, México. Orci: 0009-0007-6474-9124

Alejandro C. Monsalvo-Reyes
Laboratorio de Bioquímica Molecular Unidad de Biotecnología y Prototipos UBIPRO FES Iztacala UNAM, México. Orcid: 0000-0001-8622-6227

Ricardo E. Ahumada-Cota
Unidad Periférica de Investigación Básico y Clínica en Enfermedades Infecciosas Laboratorio de Patogenicidad Bacteriana Unidad de Hemato-Oncología e Investigación Hospital Infantil de México Federico Gómez Departamento de Salud Pública División de Investigación Facultad de Medicina UNAM, México. Orcid: 0000-0002-1636-0057

Carlos Eslava-Campos
Unidad Periférica de Investigación Básico y Clínica en Enfermedades Infecciosas Laboratorio de Patogenicidad Bacteriana Unidad de Hemato-Oncología e Investigación Hospital Infantil de México Federico Gómez Departamento de Salud Pública División de Investigación Facultad de Medicina UNAM, México. Orcid: 0000-0002-6286-6454

Ulises Hernández-Chiñas
Unidad Periférica de Investigación Básico y Clínica en Enfermedades Infecciosas Laboratorio de Patogenicidad Bacteriana Unidad de Hemato-Oncología e Investigación Hospital Infantil de México Federico Gómez Departamento de Salud Pública División de Investigación Facultad de Medicina UNAM, México. Orcid: 0000-0003-4708-8807

Abstract.: Introduction. Phage Display is a technique that enables the identification of immunodominant epitopes that can be proposed as immunogens in the development of vaccines. Liposaccharides (LPS) participate in the pathogenesis of the microorganism and induce the immune response in the host. However, due to its toxicity, it cannot be employed as an immunogen in vaccine development. **Objectives**. To select phagotopes of immunodominant epitopes of *Salmonella* Typhi LPS, evaluate if they present immunogenic properties and to analyze if the resulting antibodies against the phagotopes recognize *S*. Typhi LPS. **Materials and Methods**. Using Phage Display with a peptide library of 12 amino acids (BioLabs) and IgG from *S*. Typhi anti-LPS rabbit sera, phagotopes were selected. After obtaining DNA sequences, peptides conjugated with KLH were synthesized. The reactivity of the *S*. Typhi anti-LPS rabbit sera, anti-phagotopes and anti-peptides against LPS and peptides were evaluated. Using the same sera, S. Typhi LPS recognition was analyzed by Western blot. **Results**. Analysis of the DNA sequences of f073/2 and f073/14 phagotopes revealed the TDYA motif as a consensus sequence. Using these peptides and phagotopes, the following sera were obtained: anti-f073/2, anti-f073/14, anti-SP073/2-KLH and anti-SP073/14-KLH. Reactivity analysis of the anti-SP073/2-KLH and anti-SP073/14-KLH sera showed that both recognized *S*. Typhi LPS with an OD (415nm) of 0.35 and 0.73, respectively. Similarly, the homologous peptide was recognized with values of 0.55 and 0.65 at a dilution of 1:50. With respect to the anti-f073/2 and anti-f073/14 sera, they recognized both the LPS of *S*. Typhi and the peptides. Western blot analysis of the anti-LPS *S*. Typhi, anti-phages and anti-peptides sera showed recognition of the core region and lipid A of the *S*. Typhi LPS. **Conclusion**. The phagotopes selected

with the anti-LPS serum of *S*. Typhi showed immunogenic properties. In addition, the synthetic anti-peptide antibodies generated with the phagotopes consensus sequence recognized both the homologous peptide and the LPS of *S*. Typhi. The results suggest the possible use of mimotope peptides in the design of an immunogenic protector against *S*. Typhi infections.

Keywords: phagotope, mimotope, *S*. Typhi LPS, Phage Display.

INTRODUCTION

Lipopolysaccharide (LPS) is an important component of the cell wall of Gram-negative bacteria. The external region of the LPS is formed by repeat units of carbohydrates that provide antigenic variability to enterobacteria. Finally, a central or core part comprises polysaccharides and a hydrophobic region in which lipid A or endotoxin is located (Caroff, 2003; Leclercq, 2021). The combination of these structures helps in the pathogenicity of the microorganism and at times, activates the immune response of the host. *Salmonella* enterica serovar Typhi (*S*. Typhi) is an important pathogen both clinically and epidemiologically (Pollard, 2019). Salmonellas can infect animals and humans, and cause gastroenteritis in infants and adults, as well as systemic infections, such as typhoid fever, which is related to *S*. Typhi (Stanaway, 2019; Majowicz, 2010). Increase in the prevalence worldwide of infections caused by *Salmonella* is considerable. In 2013 in the United States of America, 19,056 cases and 80 deaths with an incidence of 15.19 per 100,000 population were reported (Crim, 2014). In Mexico in 2018, the Office of Epidemiology of Mexico reported 79,203 cases *Salmonella* no Typhi infections across all age groups (SINAVE, 2019). In the same year, SINAVE reported 34,906 cases of typhoid fever caused by *S*. Typhi representing 27.85 cases per 100,000 population. These figures highlight the clinical and epidemiologic importance of *Salmonella* and the need to implement measures to control its prevalence in both animals and humans. Among other measures, the development of vaccines that are easy to apply and without side effects for the individual is an effective option.

Smith (1985) developed Phage Display that presents phage peptides and allows specific mimotope peptides to be selected that are expressed on the surface of the filamentous M13 phage. This phage harbors a library of peptides that are expressed at random, some of which could be immunogenic epitopes of a specific antigen of interest. Previous studies have reported on the selection of mimotope peptides of the LPS of *E. coli* O157, *Vibrio cholerae* O1, O139, *Brucella* and some other microorganisms, as well as of products secreted by bacteria (Navarro *et al*, 2016; Falklind-Jerkérus, 2005; Passo, 2007; De Bolle, 1999; Dharmasena, 2007; Ulises, 2009). The procedure consists of selecting the phagotopes (phages that express peptides) with specific antibodies obtained from the sera of animals or humans. Using the selected phages, DNA is obtained and sequenced to translate the amino acid sequence of the mimotope peptide chosen. Using the sequence, synthesis of the corresponding peptide is carried out in order to evaluate if they react against the sera that were used to select the phagotopes. The mimotope peptides join to hemocyanin of the Californian limpet (Keyhole limpet hemocyanin: KLH) and rabbits immunized with the resulting antibodies were analyzed to determine if they reacted to the antigen being studies (LPS, protein, toxin etc.). If the results proved positive, the mimotope peptides were evaluated in animal models to see if they function as immunogens that activate a protective immune response against the microorganism from which the original

analysis started (Falklind-Jerkérus, 2005; Passo, 2007). Phage Display has been used with successful results in our laboratory to identify immunodominant epitopes of the serine proteases Pet and Pic secreted by *E. coli* and *S. flexneri*, as well as the LPS of *E. coli* O157, *S.* Urbana and *S.* Arizona (Ulises, 2009; Navarro, 2020). The results generated by the identification of LPS mimotope peptides of *S.* Urbana and *S.* Arizona (Navarro, 2020) allow us to envisage the possible use of Phage Display in the search of immunogens for the development of vaccines against bacteria of medical importance without the toxic effect of LPS. The aim of the following study was to select phagotopes of immunodominant epitopes of *S.* Typhi LPS and analyze their potential as immunogens, as well as the capacity of the resulting antibodies to react against the phagotopes with which they were generated and against the LPS of *S.* Typhi.

MATERIAL AND METHODS

Lipopolysaccharides and cores. The phenol-water method proposed by Westphal (1965) was used to extract and purify the LPS of *S.* Typhi and the cores of mutant strains of *E. coli*, as well as the Ra, Rd and Re cores of S. Minnesota (List Biological Laboratories, Campbell, C A, EE UU). The *S.* Typhi ATCC 6539 (FMU073) strain and the mutant strains of *E. coli*, R1 (O8:K27), R2 (O100:K?:H2), R3 (O111:K58:H, R4 (O14:K7) were obtained from the collection of enterobacteria kept by the Faculty of Medicine at the National Autonomous University of Mexico (UNAM). The LPS and cores were treated with DNase, RNase and proteinase K. Following extraction, the LPS and cores were lyophilized (Labconco, Kansas City, Missouri, USA) and kept refrigerated until used.

Production of anti-LPS sera. Before immunizing 2kg New Zealand rabbits, blood samples were taken to use as a negative control for the various tests. The rabbits were obtained from the Central Animal House of the faculty of Medicine, UNAM. The rabbits were immunized with the LPS of *S.* Typhi using the protocol described by Ewing (1986). The first dose comprised an intradermic injection of 100 mg/mL of LPS in PBS with Freund adjuvant, followed by for further doses at seven-day intervals. A week after the final dose, the rabbits were bled under anesthetic. Using 1.0 mL per kg, pentobarbital (Anestesal, Pfizer) was administered intravenously to completely anesthetize the rabbits. The care and management of the rabbits was carried out according to official Mexican norms (NOM-062-ZOO-1999).

Production of anti-phagotope sera. Two white New Zealand rabbits (1.5kg) were immunized subcutaneously at 7-day intervals and inoculated with 5 doses of the phagotope clones that were chosen using 10^{13} units formed of plates (ufp) according to the procedure described previously (Navarro *et al.*, 2016). Before immunization, rabbit sera were taken to be used as a negative control for the ELISA tests. Blood samples were taken one week after the fifth dose of the phagotope was administered. The blood samples were centrifuged at 4500 rpm and the 1mL sera aliquots were stored at -20 °C until use. Immunization was carried out in accordance with the specific techniques for the care and management laboratory animals described in the official Mexican norms (NOM-062-ZOO-1999).

Biopanning. Using the Page Display method describe by Smith (1985) and a library of peptides comprising 12 amino acids expressed in the M13 filamentous phage (New England, BioLabs) phage clones that contained mimotope peptides of the LPS were selected. With the DNA sequence of the phages, the amino acid sequences corresponding to the peptides expressed in the protein III of

the M13 phage were obtained. With these sequences, the synthesis of the peptides that were obtained in linear sequences and attached to KLH, (BioSynthesis). The synthetic peptides attached to KLH (SP-KLH) and the phage clones were used to immunize rabbits according to the immunization scheme reported previously (Galfré, 1996).

Production of mimotope anti-peptide sera. New Zealand white rabbits (1.5kg) were immunized at 7-day intervals and inoculated with 5 doses of the peptides SP073/2 and SP073/14 conjugated with KLH. The priming dose was 500 µg followed by 4 doses of 1.0 mg according to the previously described method (Galfré, 1996). Before immunization, serum samples were taken from the rabbits to use as negative controls. One week after immunization, the rabbits were bled and the aliquots were stored at -20 °C until use. Blood samples were taken under anesthetic using pentobarbital (Anestesal, Pfizer) was administered intravenously to completely anesthetize the rabbits. The care and management of the rabbits was carried out according to official Mexican norms (NOM-062-ZOO-1999) to obtain the production of anti-LPS and anti-phagotope sera.

Evaluation of anti-phagotope and anti-peptide reactivity against peptides, cores and LPS using ELISA. The capacity of anti-f073/2, anti-f073/14, anti-SP073/2-KLH and anti-SP073/14-KLH sera to react against the SP073/2 and SP073/14 peptides, cores and LPS was analyzed by ELISA as described previously (Navarro *et al.*, 2016). Briefly, 96-well microplates were used (Nunc-Immuno Plate, MaxiSorp F96) into which the peptides, cores and LPS that had been dissolved beforehand in a carbonate buffer pH 9.6 were placed. The plates were incubated at 37 °C for 2 h and then at 4 °C for 18-24 h. The plates were then blocked with 200 mL of 1% PBS low fat milk (Svelty, Nestlé) at an ambient temperature for 2 h, and then washed three times with 0.05% PBS/Tween 20. Double serial dilutions were carried out at a ratio of 1:50 and up to 1:1600 of the anti-phagotope and anti-peptide sera in PBS with a pH of 7.4 and incubated at 37 °C for 2 h. The plates were washed three more times (PBS/Tween 20) and 100 mL of rabbit anti-IgG (1:1000) conjugated with alkaline phosphate (Invitrogen, USA) were added to each well. The plates were incubated at 37 °C for 2 h and the reaction observed by adding 200 µL of p-nitrophenyl phosphate (1 mg/mL, Sigma) in diethanolamine buffer (pH 9.8, Sigma). The reaction was stopped by adding 25 µL of NaOH (3M). An ELISA reader (BioTek ELx800) adjusted to 415 nm was used to read absorbance. All of the tests were carried out independently in duplicate. The sera from preimmunized rabbits were used as negative controls for reactivity.

Recognition of LPS and cores by anti-phagotope and anti-peptide sera. Western blot (Laemmli, 1970) was used to identify the interaction site of the anti-f073/2, anti-f073/14, anti-SP073/2-KLH and anti-SP073/14-KLH sera with the *S.* Typhi LPS, as well as the Ra, Rb, Rd and Re cores of *Salmonella* Minnesota (List Biological Laboratories) and the R1, R2, R3, R4 and K12 cores of *E. coli*. Using SDS-PAGE a polyacrylamide gel (15%) with 4 M urea, 10 µL samples of the LPS and cores were separated and transferred onto nitrocellulose membranes, The reaction was observed using rabbit anti-IgG labeled with alkaline phosphatase (Invitrogen).

RESULTS

Selection of phagotopes with mimotope peptides. Using Phage Display, 15 -TDYAFS motif as a consensus sequence (Table 1). Synthesis of the peptides SP073/2 and SP073/14 were obtained in linear sequence and conjugated with KLH, SP073/2 and SP073/14.

Reactivity of the anti-phagotopes f073/2 and f073/14 sera against LPS and cores by ELISA. Two anti-sera against the phagotope clones f073/2 and f073/14 were obtained. The anti-phagotope f073/2 reacted against the homologous peptide SP073/2, *S.* Typhi LPS and the R1, R3 and Ra cores at a dilution of 1:100 with an absorbance (415nm) of 0.71, 0.70, 0.79, 0.32 and 0.22, respectively. With regards to anti f073/14 serum, it reacted against the homologous peptide SP073/14, *S.* Typhi LPS, R4 core and R3 core at a dilution of 1:100 with absorbance values of 0.11, 0.42, 0.94 and 0.12, respectively (Table 2).

Reactivity of the SP073/2-KLH and SP073/14-KLH anti-peptide sera. The serum against SP073/2 and SP073/14 peptides showed that anti-SP073/2-KLH reacted against *S.* Typhi LPS, the SP073/2 peptide, the R1, R2 and Ra cores at a 1:100 dilution with absorbance (415nm) values of 0.52, 0.37, 1.06, 0.41 and 0.31, respectively (Table 3). Considering the SP073/14-KLH serum, it reacted against *S.* Typhi LPS, the homologous synthetic peptide SP073/14, and the R2, R3 and Ra cores with values of 0.59, 0.34, 0.46, 0.22 and 0.32 (Table 3).

Recognition of peptides SP073/2 and SP073/14 by anti-core sera. In an assay to analyze the reactivity of anti-lipid A, anti-R1, anti-R3 and anti-Ra sera at a 1:50 dilution against the SP073/2 peptide, reactivity was observed for each one with absorbance values of 0.7, 0.64, 0.63 and 0.29 respectively. The same test but evaluating the response against the peptide SP073/14, it was observed that it was recognized by anti-lipid A, anti-R3, anti-R4, anti-Ra and anti-Re sera with absorbance values of 0.49, 0.64, 0.49, 0.3 and 0.56 respectively.

Analysis of the response against *Salmonella* and *E. coli* antigens by anti-phagotope and anti-PS sera by Western blot. Western blot showed the reactivity of anti-fSP073/2 and anti-fSP073/14 sera against the lipid A region and core of S. Typhi (Figure 1). With respect to the anti-f073/14 serum, it recognized the K12, R1, R2, R3, R4, Ra and Re cores, with the last two being *Salmonella* (Figure 2). The same analysis for anti-SP073/2-KLH serum showed a response against the R1 and R2 cores (Figure 3). Meanwhile, anti-SP073/14-KLH serum mainly recognized the R3 core (Figure 4).

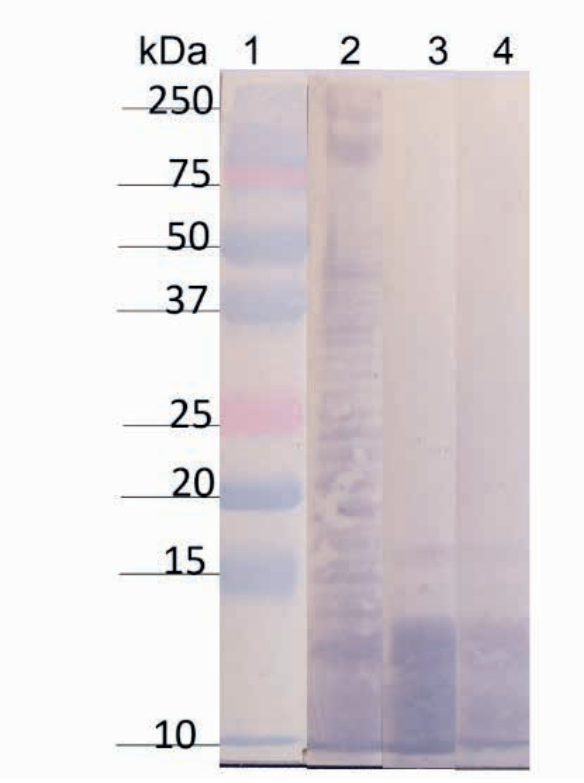

Figure 1. Antibody response of *S.* Typhi anti-LPS, anti-f073/2 and anti-f073/14 sera against purified *S.* Typhi LPS. Western blot was performed using the sera at a 1:50 dilution. Lane 1: molecular weight marker; lanes 2, 3 and 4, LPS of *S.* Typhi. In lane 2, recognition of the *S.* Typhi LPS ladder pattern by anti-*S.* Typhi LPS serum is observed; lanes 3 and 4 show recognition of the lipid A region and the core region by the anti-f073/2 and anti-f073/14 sera. This was considered a positive reaction regardless of intensity.

Peptide code	Amino acid sequence																				Molecular weight	Selected clones
PS073/2*	S	V	E	T	D	Y	A	F	S	N	D	X	X	F							1394.42	10
PS073/4							S	F	F	P	A	X	X	F	T	N	P	Q	L	N	1273.41	3
PS073/14						I	S	L	S	N	I	V	D	S	Q	T	P				1382.59	2

Table 1. Amino acid sequences and alignment of the selected phagotopes using Phage Display.

Consensus Sequence DXSFSN/F/P

*10 clones were selected with a similar sequence to SP073/2, the consensus motif was TDYAFS.

Dilution	Anti-phage f073/2 Serum					Anti-phage f073/14 Serum			
	Antigens					Antigens			
	SP073/2	LPS 073	R1	R3	Ra	SP073/14	LPS 073	R4	R3
1:50	0.89	0.85	1.27	0.46	0.35	0.18	0.57	1.13	0.13
1:100	0.71	0.7	0.79	0.32	0.22	0.11	0.42	0.94	0.12
1:200	0.55	0.47	0.47	0.24	0.15	0.08	0.24	0.64	0.08
1:400	0.42	0.24	0.28	0.18	0.1	0.04	0.15	0.41	0.07
1:800	0.31	0.13	0.19	0.14	0.08	0.04	0.07	0.26	0.05
1:1600	0.2	0.13	0.16	0.09	0.08	0.03	0.04	0.17	0.05

Table 2. Response of the anti-Φ073/2 and anti-Φ073/14 sera against the S. Typhi LPS, mimotope peptides and cores of E. coli and Salmonella (10 μg/mL).

Optical Density (OD) readings 415 nm. 073: S. Typhi.

Dilution	Anti-SP073/2-KLH serum					Anti-SP073/14-KLH serum				
	Antigens					Antigens				
	LPS 073	SP073/2	R1	R2	Ra	LPS 073	SP073/14	R2	R3	Ra
1:50	0.35	0.55	1.82	0.62	0.37	0.73	0.65	0.66	0.25	0.32
1:100	0.31	0.37	1.06	0.41	0.31	0.59	0.34	0.46	0.22	0.32
1:200	0.21	0.22	0.67	0.33	0.17	0.32	0.18	0.25	0.21	0.25
1:400	0.15	0.16	0.38	0.25	0.14	0.19	0.09	0.16	0.17	0.16
1:800	0.1	0.11	0.22	0.1	0.04	0.11	0.05	0.09	0.17	0.10
1:1600	0.1	0.07	0.16	0.06	0.03	0.07	0.04	0.06	0.15	0.06

Table 3. Response of the anti-SP073/2-KLH and anti-SP073/14-KLH sera against LPS O73, homologous peptides and cores (10 μg/mL).

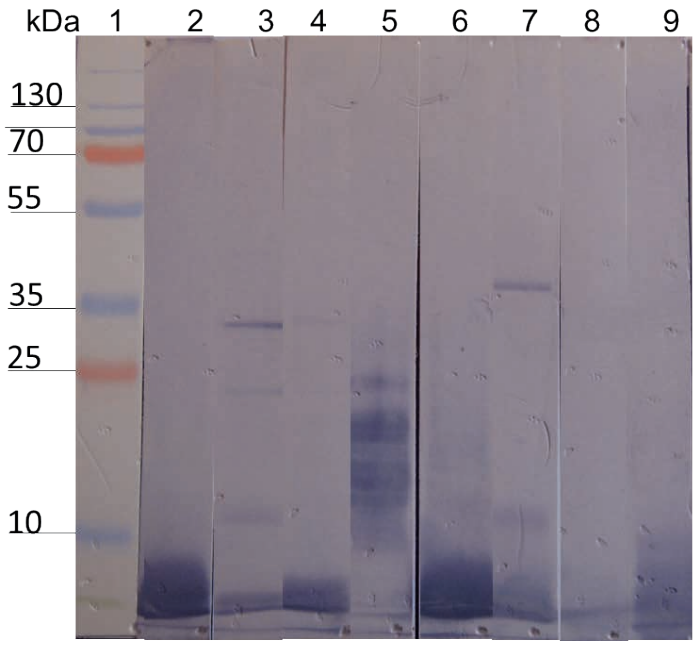

Figure 2. Anti-phage f073/14 antibody response against *E. coli* and *Salmonella* cores. Western blot was performed using the purified cores. Lane 1: molecular weight marker; lanes 2, 3, 4, 5, 6, 7, 8 and 9, cores K12, R1, R2, R3, R4, Ra, Rd and Re, anti-f073/14 serum was used at a 1:50 dilution. Recognition of the regions of the cores K12, R1, R2, R3, R4, Ra, Rd and Re. This was considered a positive reaction regardless of the intensity.

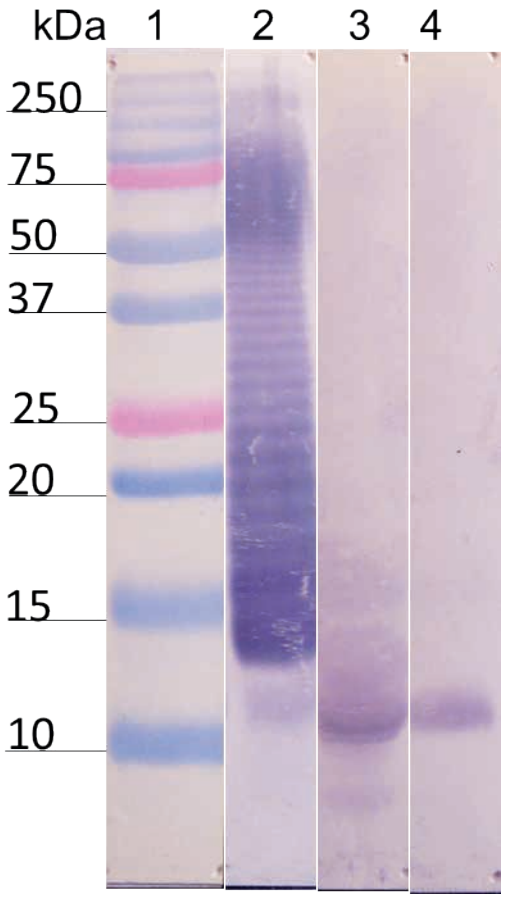

Figure 3. Anti-SP073/2-KLH serum antibody response against cores R1 and R2 of *E. coli*. Western blot was performed using the LPS of *S*. Typhi and purified cores; sera were used at a 1:50 dilution: Lane 1: molecular weight marker; lanes 2, 3 and 4, S. Typhi LPS, cores R1 and R2 of *E. coli*. In lane 2, recognition of the *S*. Typhi LPS ladder pattern by the S. Typhi anti-LPS serum; In lanes 3 and 4, recognition of the R1 and R2 cores of *E. coli* by the anti-SP073/2-KLH serum. This was considered a positive reaction regardless of the intensity.

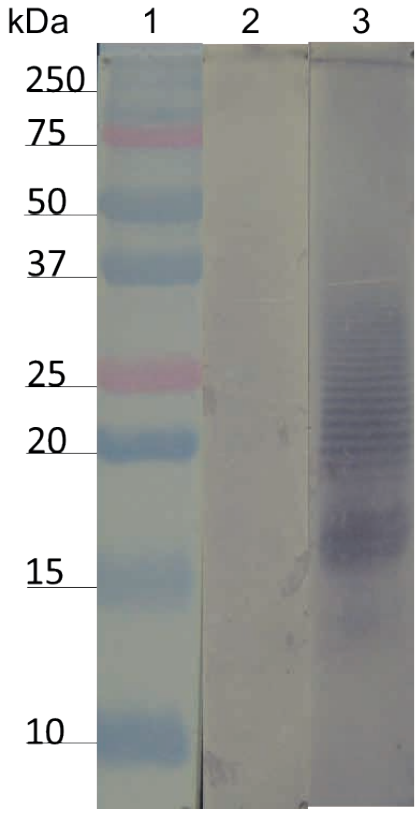

Figure 4. Response of anti-SP073/14-KLH antibodies against core R3 of *S*. Typhi. The Western blot was performed using the purified core. Lane 1: molecular weight marker; lanes 2 and 3 core R3, preimmunized serum and anti-SP073/14-KLH serum were used at a dilution of 1:50. Recognition of the R3 core fractions, this was considered a positive reaction regardless of intensity.

DISCUSSION

The overuse of antimicrobials in the treatment of infectious diseases in humans and animals and their use in the agroindustry has been a major reason for the increase in bacterial resistance to these compounds moving us closer to a significant public health problem (Miranda-Novales, 2020). The WHO states that the pathogenic principals of resistance to antibiotics (https://www.who.int/news/item/27-02-2017) are included in 12 families of bacteria with a big impact on human health. Among these bacteria, some have been defined as being of high and medium priority

including bacteria that show increasing drug resistance and provoking common diseases, such as gonorrhea and foodborne intoxication by *Salmonella*. Although the WHO suggests searching for new antimicrobials to control resistance, maybe there should be measures put in place to manage the appropriate use of existing antimicrobials or those new ones that are on the increase. A better alternative could be the development of ways to prevent infectious diseases. Salmonellosis is a zoonotic infectious disease transmitted via a large range of foodstuffs. Reservoirs of *Salmonella* (*S*. Typhimurium y *S*. Enteritidis) include domestic and wild animals, such as poultry, pigs, cattle, rodents and pets such as iguanas, turtles, dogs, cats, and hamsters, as well as human beings (Percival, 2014). S. Typhi is another variety of *Salmonella* and an etiological agent of typhoid fever, which is a potentially deadly infections disease and like other salmonellas is transmitted by water or contaminated food. *S.* Typhimurium and *S.* Enteritidis are microorganisms related to diarrheal disease transmitted by food and annually, 94 million cases and approximately 155,000 deaths are reported. Figures for *S.* Typhi show annual cases to be between 11.9 and 26.9 million that lead to between 128,000 and 216,500 deaths (Daigle, 2021). Treatment for *Salmonella* infection centers around antimicrobials but resistance is complicating the management and control of cases (Karkey, 2018; Emond-Rheault, 2020). Although vaccines exist for preventing and controlling *Salmonella* infections, they are mainly used in animals. There is a specific conjugated vaccine against *S.* Typhi that although offers more prolonged immunity, its use is restricted to certain areas (Gibani, 2018). Considering the fact that treating *Salmonella* is becoming more and more complicated and having a biological available to control the infection without potential collateral effects, which have been reported about vaccines being used currently, it is necessary to look for alternative ways to treat and control *Salmonella* infections. Phage Display is a system that expresses peptides at random in protein III of the M13 filamentous phage (Smith, 1985). Some of these peptides could be mimotopes of polysaccharide or protein epitopes, as well as other molecules of the microorganisms that could be used as possible immunogens for the development of vaccines (Aghebati-Maleki, 2016; Fukuda, 2011; González-Mora, 2020). The natural or innate response is the first line of general defense by the body to pathogens, which is activated by capturing signals emitted by viral or bacterial components or by proteins liberated by the pathogen. This capture is handled by pattern recognition receptors (PRR) that identify foreign compounds as they enter the body through pathogen-associated molecular patterns (PAMP) and damage-associated molecular patterns (DAMP). Toll-like receptors (TLR) that recognize bacterial and viral products form part of the PRR. Meanwhile, some reports suggest that the phage genome contains CpG nucleotides that allow interaction with LPS (Hess, 2019). It is this combination of elements that provide phages with the potential of being candidates for the development of vaccines, either in their own right or as carriers of antigens with immunogenic capacity (Hess, 2019).

A previous study (Navarro, 2020) using Phage Display identified mimotope peptides of the LPS of *E. coli* O157. The serum obtained in rabbits inoculated with the mimotope peptides recognized the *E. coli* O157 LPS and the respective one from *S.* Typhi, *S.* Urbana, and *S.* Arizonae. With the aim of finding specific epitopes against *Salmonella*, our current study used the same Phage Display method to select mimotope peptides from immunodominant sites of the LPS using

serum from rabbits challenged with *S*. Typhi LPS. Of the 15 phagotopes obtained, DNA sequencing analysis showed that 10 of them possessed the TDYAFS consensus sequence. One sequence with a similar motif (DYAW) reported by Passo (2007) was used to identify carbohydrate mimotope peptides of the capsule from *Neisseria meningitidis* of the B serogroup. The difference between these motifs (DYAF and DYAW) was that in the amino acid sequence in the current study, phenylalanine aromatic amino acid (F) was found in place of tryptophan (W). The DYA trimer reported by Shi (2018) was present in a peptide of an epitope of the Fructose-diphosphate aldolase enzyme and when administered to rats, it showed protective effects when challenged with *Candida albicans*. The consensus sequence identified in our current study showed the presence of Tyr and Ser amino acids that were reported in the composition of carbohydrate mimotope peptides of cell wall structures in different microorganisms (Harris, 1997; Oldenburg, 1992; Agostino, 2011). Shin (2002) referred to the presence of phenylalanine as an important component in mimotope peptides of the polysaccharide capsule of *Streptococcus pneumoniae*. The importance of certain amino acids in the composition of mimotope peptides is corroborated by Smith (2009) who reported that rats immunized with mimotope peptides, which carried a dimer of lysine and phenylalanine (KF), were not infected when the animals were challenged with a culture of *S. pneumoniae*. In another study, Thomas (2003) identified mimotope peptides of lipid A of enterobacteria in which the most abundant amino acids were phenylalanine (F), tyrosine (Y) and tryptophan (W). In our current study, the amino acids identified in the sequence of SP073/14 showed phenylalanine forming a dipeptide con proline (FP) and with serine (FS). This suggests that these amino acid residues in the mimotope peptides reported in this study could be related to the recognition of *S*. Typhi LPS and cores by anti-*S*. Typhi mimotope sera.

The mimotope peptide SP073/14 presents serine (S) that together with proline (P), phenylalanine (F) and tyrosine (Y) have been identified in mimotopes of carbohydrate structures of the cell wall of several microorganisms (Agostino, 2011). In this same peptide (SP073/14), other dipeptides made up of asparagine-isoleucine (NI) and isoleucine-serine (IS) were also found, while in peptide SP073/2 the FS dimer (phenylalanine, serine) was identified. In a study reported by (38) Wu (2010), the dipeptides NI and isoleucine- threonine (IT), mimotope peptides from the capsules of *Streptococcus agalactiae* and *Neisseria meningitidis*, were present. Immunization of animals with peptides with this motif induced the production of antibodies against the capsule of these microorganisms, and also against other Gram-positive bacteria. Additionally, the two dipeptides mentioned (NI and IT) were reported in another study (Navarro, 2020) concerning mimotope peptides of the LPS of *E. coli* O157 and *Salmonella*, in which the anti-mimotope sera recognized the R1 and R3 cores of *E. coli*. It may be that the presence of the dipeptides NI and IS in the synthetic peptide SP073/14 could be related to the production of antibodies against the LPS evaluated in our study.

It is important to highlight the Western blot results relating to anti-phage sera f073/2 and f073/14 that recognized the region corresponding to lipid A of *S*. Typhi. A similar response was observed in the ELISA assay, in which both sera recognized both their homologous peptides (SP073/2 and SP073/14), as well as the LPS of *S*. Typhi, the R3 and R4 cores of *E. coli* and the Ra core of *Salmonella*. A possible explanation for the reactivity of

anti-phage sera against the cores is the fact that the Ra core of *Salmonella* is similar to the R2 core of *E. coli* (Raetz, 2002; Jansson, 1981). *E. coli* cores (R1-R4) are composed of 5 hexoses, while the *Salmonella* and R3 cores are made up of glucose-galactose-glucose trisaccharide (Jansson, 1981). The recognition of a core by the anti-phage sera could be due to the structural similarity that these regions of the LPS of *E. coli* and *Salmonella* present. In addition to this, Hess (2020) suggested that the phage genome contains CpG nucleotides that allow it to interact with LPS.

Another interesting observation from the study was that the SP073/2 and SP073/14 peptides were recognized by the anti-peptide sera, as well as the anti-LPS, anti-Re and anti-Ra of *Salmonella*, and anti-lipid A, anti-R3, anti-R4. Van Regenmortel (2009) and Muller (2001) proposed that a peptide can be considered a mimotope of an epitope when the antiserum prepared against the peptide in question reacts with the original immunogen (LPS of *S*. Typhi). This is why SP073/2 and SP073/14 peptides can be considered as mimotopes of the immunodominant epitope of the core and of *S*. Typhi LPS.

In concurrence with González-Mora, (2020), the selected peptides in our study may be mimicking epitopes either functionally or by their conformational structure, which coincides with the carbohydrates in the core region of *S*. Typhi LPS. The results suggest that the SP073/2 and SP073/14 peptides selected by Phage Display probably present a conformational structure that resembles the conformational structure of *S*. Typhi LPS. This structural similarity confers immunogenic properties that induce the formation of antibodies, which react with *S*. Typhi LPS. For this reason, SP073/2 and SP073/14 peptides that are mimotopes of *S*. Typhi LPS could be utilized in the design of vaccines against the bacterium without the usual toxic effect of LPS.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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