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Review Article

Evaluation of SicA gene elimination from Salmonella enteritidis as a candidate for gene vaccine

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ABSTRACT:

Salmonella is a facultative intracellular pathogen which is serotype and host-dependent and can cause diseases from brain inflammation to typhoid fever. SicA is one of the virulence genes of Salmonella. SicAcause activation of the transcription from SigD/sopB and sopE that code the secreted effective proteins encoding which cause increased invasion. The purpose of this study is creation of a gene quasi-structure for the elimination of SicA gene from Salmonella enteritidis. SicA gene of Salmonella enteritidis bacterium causes the elimination of this gene. This gene quasi-structure can be used for preparation of a weak strain as a candidate for gene vaccine against Salmonella enteritidis in the future researches.

Key words: Salmonella enteritidis, SicA gene, gene vaccine

INTRODUCTION:

Salmonella is a facultative intracellular pathogen which is serotype and host-dependent and can cause diseases from brain inflammation to typhoid fever. Salmonella is a gram-negative, anaerobic bacterium which is belonged to the Entrobacteriaceae family. Salmonella genus consists of two species, S.enterica and S.bongari that the most pathogen species of Salmonella which cause diseases in human beings are belonged to S.entericasp which live in gastrointestinal system of the host; certain serotypes of Salmonella have particular host and some others have general host (1). S. enteritidis and S. typhimorium are the cause of 24.1% and 6.1% of the common food poisoning in the world, respectively. Both serotypes are entered by eating the contaminated food and contaminated water. The most common foods related to Salmonella are the foods with animal origin; some foods which are related to some serotypes of Salmonella strongly have been identified. The most famous of them is egg which is related to S. enteritidis and is the reason of 73.7% of food poisoning in the world. This bacterium (S. enteritidis) has two types of flagella which are involved in the connection of S. enteritidis to the epithelial cells of the small intestine and mouth. The ability to enter and survive in the host cells is a prerequisite for the pathogenicity of Salmonella sp..The invasion of Salmonella bacteria to the epithelial cells depends on the sensitive changes of the cell including reorganization of actin filaments of the cytoskeleton and membrane stimulation to produce the necessary changes in the cell

membrane (2). Salmonella produces its effective proteins through the secretion system of type III and use this system to enter its virulent factors to the host cell. S. enterica is coded in less than two systems, one in sentisome 63 which needs the secretion system type III for the reaction of Salmonella with intestinal epithelium and the other in sentisome 31 which appears for the systemic infection if required (3,4). Pathogenicity of Salmonella needs to have virulence genes which some of these genes were identified on the Salmonella pathogenicity islands (SPI) of S. typhimorium chromosome, that have less than five pathogenicity islands.

Type I of Salmonella pathogenicity island (SPI) has chaperones including SicA and proteins SipB, SipA, SipD, SipCwhich are the initial need for bacterial penetration to the intestinal epithelial cells and often join the genes of tRNA, while SIP2, 3, 4 are the initial needs for the growth and survival of bacteria in the host cell. The proteins of SipA, B, D, C are the first basic characteristic of involvement of the intestinal epithelial cells (4). SicA is one of the Salmonella's chaperones which is secreted through the secretion system type III. This protein has a role in the translation process directly and contaminates the cytosol of the host cell. SicA and InvF also cause activation of the transcription from SigD/sopB and sopE that code the secreted effective proteins encoding which cause increased invasion. The expression of proteins sipC, sipC, sipB depends on the expression of gene SicA. If it is not expressed, Salmonella will lose its aggressive mode (5,6).

Lin M et al (1996) in Taiwan isolated 121 Salmonella samples from 2983 hunt sparrows around the poultry farms (7).Shareef et al (1997) in their study in Iraq have isolated S. typhimoriumfrom 6% samples of the local egg shells (8).All viral plasmids of Salmonella have a very close relationship with themselves, Chu et al in 1999 succeeded to do two large eliminations from the operon pef and the region of tra in pSCV plasmid. These eliminations and variations may be associated with adaption of Salmonella to the host cell (7)). Nagappa et al (9) isolated S. typhimorium from the eggs and meat in Tariaregion from Uttaranchal. 100 samples of egg and meat were studied and identification and verification of the operation was performed by PCR reactions. The results showed a high contamination with S. enterica and S. typhimorium and in addition to that, the sensitivity to the antibiotics Chloramphenicol, Colisitin, Polymyxin, Enrofloxacin and Ciprofloxacin was studied, too (9).

Testing method:

Preparation of standard strains of bacteria

Salmonella entericaserovarenteritidis (standard strain) was prepared from the Microbiology department of Pasteur Institute of Iran and was used in Biotechnology Research Center of Azad University, branch of Shahr-e-Kord. Also, Ecoli strain Top10 which is used for the purpose of gene cloning and recombinant plasmids replications was prepared from the Biotechnology Research Center of Azad University, branch of Shahr-e-Kord.

Master Mix was prepared at first to do PCR. For this purpose, 40 µl of MgCl2 (50mM) was poured into a clean tube and 20 µl dNTP (10mM) and 100 µl PCR buffer (10X) were added and was reached to 1000 macro-liter by water injection.

This is important that the PCR solutions must be completely thawed and mixed. Because, if the solutions are not completely thawed, different melting of ingredients causes not to add to PCR with correct concentrations. The components of PCR were poured into Master Mix tube and 20 µl of it was used for each sample. Also, 1 µl of each forward & reverse primers and 3 macro-liters of template DNA were added to the tube of 20 µl Master Mix. On the last stage, TaqDNA Polymerase was removed from the freezer and added to the above tube in 0.2 macro-liters and then returned to the freezer.

Then, the samples were placed in the thermo-cycler and PCR was performed according to the thermal program of table 1.

Table1-thermal cycles in PCR, annealing temperature for each three genes were shown in stage 2.

stage	temperature	time	Number of cycles
Prog1:Initial denaturatin	95	5 min	1
Prog2: Seg1:Denaturion Seg2:Annealing Seg3:Extension	 94 58-61 72	 1 min 1 min 1 min	32
Prog3:Final Extension	72	5 min	1
Hold	10	5 min	1

PCR amplification on Up & Down regions of SicAgene of S. enteritidis

To replicate upstream and downstream regions of the intended gene, PCR was used. To do this, forward and reverse primers for both gene regions which are given in table (3-3) were used. To facilitate cloning, the cutting sites of Xbal and KpnI enzymes were placed near the 5' head of each primersSicA-up-F and SicA-up-R which are known by drawing a line under them. The product of replication reaction was 447 bp.To replicate the end region of SicA gene, SicA-dwn-F and SicA-dwn-R which have the enzymatic cutting sites of SacI and XhoI respectively, were used. The length of PCR product in this reaction was 245 bp.(3)

Test Results:

Standard strain of S. enteritidisbacteria that was prepared from the Microbiology department of Pasteur Institute of Iran, was cultured on LB agar medium, successfully.

Figure 1- S. enteritidis culture on LB agar medium

The production and replication results of SicA-Down, Sic-up genes

SicA-up and SicA-down genes were replicated through the designed primers. The length of intended band for SicA-up gene is 447 bp and the annealing temperature is 61ºc for that.

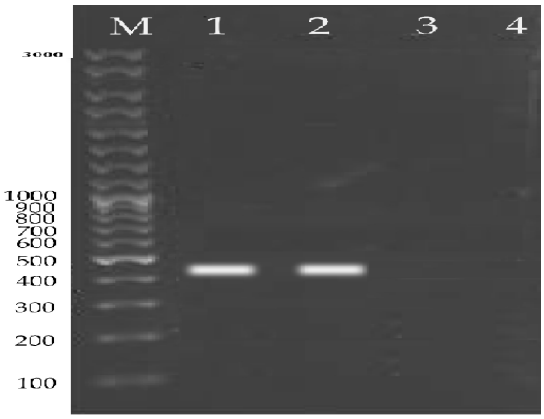

Figure 2- 447 bp band related to SicA-up gene
M: 100 bp market manufactured by Fermetase
1-2: PCR products of SicA-up gene
3: Negative control

The length of intended band for SicA-down is 245 bp and its annealing temperature is 58ºc.

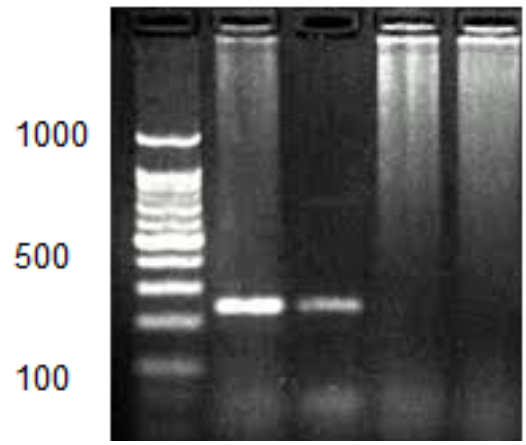

Figure 3- 245 bp band related to SicA-Down
M: 100 bp market manufactured by Fermetase
1-2: PCR products of SicA-down gene

DISCUSSION AND CONCLUSION:

In the study of Higashide et al (2002) some tests were performed within a living organism that had been infected by Salmonella to analyze SipA gene and they observed that the expression of this gene affects on the peripheral actin filaments and increase their polymerization (11). In this experiment, it was

found that in the infected cells by Salmonella whichhave mutation in its SipA gene, the rate of infection was reduced about 80% (11). In another study by Lilic et al (2003) with the help of crystallography of X-ray from SipA gene and re-organization of electron micrographs from the actin filaments and then modeling and mutagenesis on its structure, showed that SipA gene is involved in the stability of actin filaments as a main molecular component (10). Taraki et al (2005-2008) collected the samples of S. kentuchy from various resources in Tunesia and studied their antimicrobial resistance. Their studies showed that the ability of biofilm formation in Salmonella is related to the presence of virulence genes such as invA, spv C and sdi A (12).Another study in 2012 in Italy by Bacci et al was conducted by PCR method on antibiotic resistance of S. enteric sp. isolated from the poultry meat. In this research, it was found that the isolated species are resistant to the antibiotics of Ampicilin, Gentamicin, Sulfamethoxazole and Tetracycline. Also, it was showed that the cause of resistance of the species to these antibiotics, is the presence of the genes pes 1, ant (3")-la, qacEΔI, sul-1 and tet A, B, G (14). Asakura et al (2012) studied membrane topology changes of the protein SipB of S. typhimurium. Their studiesfound that this protein shows membrane topology nature in the bacterial osmolarity and also it was showed that the destruction of SipB and invG genes cause the reduced osmolarity of S. typhimuroim. The chemical analysis showed that increased osmolarity of sodium and potassium cause the increased membrane topology of the protein SipB and in the presence of neutralizing antibodies of this protein, the osmo-tolerance rate in the bacteria is reduced (13). This study was conducted based on the elimination of one of virulence genes in Salmonella enteritidis bacterium.

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