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Creation of gene organisms in starting and ending regions of SicA gene of Salmonella enteritidis in pGEM vector

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Abstract

Salmonella enteritidis is the most common cause of gastroenteritis. Sic A and Inv F activates transcription of sopB / sigD and sopE which coding effectively secreted proteins that enhance the code to be offensive. In this study upstream and downstream regions SicA gene of Salmonella enteritidis in pGEM vector was performed. In this study, the upstream and downstream genes SicA Salmonella enteritidis amplified based on specific primers and PCR and The fragment DNA cloned by T/A coloning method in pGEM vector and this constarct transformed into E. coli. The upstream and downstream SicA genes of Salmonella enteritidis was confirmed using PCR. The result of next steps showed that these regions were successfully cloned in E. coli. Confirmation of this conestract was done by digestion of restriction enzyme. According to the results ,can produce the conectruction via the recombination homologous and insertion of anti- biotic resistant genes between the upstream and downstream regions of SicA gene of Salmonella eneritidis as a candidate for gene vaccina against Salmonella eneritidis in future studies. Present study indicates the successful cloning of starting and ending encoder regions of SicA gene of Salmonella enteritidis in E. Coli bacteria. Thus, it seems that the structure produced in this study can be used as a gene vaccine candidate against Salmonella in future studies.

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Introduction

Salmonella enteritidis has two types of flagella that are involved in attaching the bacteria to epithelial cell of the small intestine and mouth. The ability to enter and survive in the cells of the host is the necessary requirement for pathogenicity of Salmonella species. Intestine's epithelial cells are among the cells that are not naturally . The invading power of Salmonella is dependent on a 40kb area on the chromosome which is located in Centisome area and is called Salmonella Pathogenecity Island. Salmonella are intracellular pathogens in warm blooded and cold blooded animals and zoonotic agents (Jacobsen *et al*, 2011, Figueira and Holden, 2012). Salmonella are bacterial, gram-negative, anaerobic, moving, rod shaped pathogens belonging to Enterobacteriaceae family (Swearingen *et al*, 2012, Figueira and Holden, 2012). This microorganism is transmitted by fecal oral transmission and causes gastroenteritis infection. The genus Salmonella is divided into two species: Salmonella enterica and Salmonella bongori. Most species of Salmonella pathogens that result in disease in human belong to Salmonella enterica that live in the digestive system of the host. Some Salmonella serotypes have specific host and some other have general host. Salmonella enteritidis and Salmonella typhimurium are the cause of common food poisoning in the world.

Both serotypes (Salmonella enteritidis and Salmonella typhimurium) are the result of eating contaminated food or drinking contaminated water. The most common foots associated with Salmonella are foods with animal origin. The most famous of them is egg which is associated with Salmonella enteritidis. Salmonella enteritidis has two types of flagella that are involved in attaching the bacteria to epithelial cell of the small intestine and mouth. The ability to enter and survive in the cells of the host is the necessary requirement for pathogenicity of Salmonella species. Intestine's epithelial cells are among the cells that are not naturally phagocytizing (Zou *et al*, 2012). The genes that are in SP1-1 area encode a type III protein secretion system (TTSS-1) and several effector proteins and chaperones that result in the transmission of Salmonella into host cell cytoplasm and destroy the host cell (Ly KT *et al*, 2007, Darwin K.H and Miller, 2007). Hill a gene is a central transcription regulator from invasive genes that is in Pathogenicity Island (SP-1), that encodes structural components, chaperones and effective factors of type III secretion system for the invader. Hill A is attached to pgr and INV/SPA promoters that are in "Hill A boxes" that apparently express Sic A, Hill A and Inv F (Bradley, 2005). Sic A and Inv F result in the activation of transcription from SOPb/sigD and sopE which encode the secretion effectors proteins that result in the increase of invasion (Temme, 2008). SipB, sipC, sipA protein expressions are dependent on SicA gene expression that in the case of the lack of its expression, Salmonella lose their invasive state. Objective: cloning the starting and ending regions of SicA gene of Salmonella enteritidis. The aim of this study is effective in reducing in getting infected to Salmonella infections and in creating a vaccine.

Methods and materials

Exaction of genomic DNA

The genomic DNA of Salmonella enteritidis bacteria was extracted using DNA extraction kit made by Iran Sinagene (DNP™ Kit) based on kit instructions.

Polymerase chain reaction

For sequence amplification, two gene regions were amplified using PCR method. First the starting region of SicA gene was amplified using the following primers.

SicA-up-F: 5'- TGTTCTAGACCCCACGCATATTACG -3'
SicA-up-R: 5'- CATGGTACCCTCCTGTTATCTGTC ACCG -3'

In this study, for facilitating cloning, near the '5 head, each of the SicA-up-F and SicA-up-R were placed with the order of cutting site of Xbal and Kpln

enzymes that have been underlined. The product of proliferative reaction was equal to 245 base pairs For proliferation of the ending region of SicA gene, SicA-dwn-F and SicA-dwn-R primers that have cutting sites of kpnl and sacl respectively were used. The length of PCR product in this retains was equal to 245 base pairs.

SicA-dwn-F: 5'- AGACAGAGCTCCACAGTGAACAAG -3'
SicA-dwn-R: 5'- TGTCTCGAGCCACCGTATTAAT AGCGC -3'

Polymerase chain reaction was done in the final volume of 25 microliters that contained 5.2 microliters of DNA template, q microliter of F and R primers, 200 micromoles of dNTP Mix, 5.1 micromole of MgCL2, 5.2 micromoles of buffer PCR(10X)and 1 unit of polymerase Smar Taq enzyme. Then, for preventing contamination and evaporation, 1 to 2 drop of sterile mineral oil was added to the reaction mixture.

PCR reaction was done in Mastercycler Gradient Eppendrof thermosycler made in Germany with thermal conditions of 5 minutes initial denaturation at 95 degrees Celsius and continuation of 32 cycles including denaturation at 95 degrees Celsius for 1 minute, bond for primers of starting region at 61 degrees Celsius and at 58 degrees Celsius for ending primers for 1 minute and elongation was done at 72 degrees Celsius for 5 minutes.

Extraction of DNA from gel

The PCR product with 1 percent gel having etidium bromide was electrophoresed. The DNA part related to SicA gene was cut by scalp razor and was extracted from gel by the DNA extraction kit made by BIONNER Company of South Korea based on the kit instructions. To ensure the accuracy of the extracted part and its quality, 3 microliter of it on 5.1 percent agarose gel was studied with electrophoresis marker.

d. T/A cloning: for doing this, the PCR product extracted from gel was cloned using a kit (TOPO TA Cloning Kit) from Invitrogne company made in United States. First, the (Ligation) attached product was transformed in E.coli HB101 and the attached bacteria was cultured in LB solid media containing amipicillin (100 micrograms in each milliliter) and was kept in 37 degrees Celsius incubator for 18 hours. Plasmid extraction was done from the grown colonies with the aid of Plasmid purification kit of Qiagen Company made in the United States. And then with PCR method, verification of cloning was done. The final verification of the obtained organism was done using enzyme digest method.

Results and discussions

DNA electrophoresis

In this study genomic DNA was successfully extracted from Salmonella enteritidis. The result of extracted DNA electrophoresis on agarose gel indicated its good quality for molecular tests. Polymerase chain reaction using specific primers of starting and ending regions of SicA gene resulted in the proliferation of parts with the lengths of 447 and 425 base pairs. Cloning PCR products was used with T/A method in pGEM vector for creating gene organism from SicA gene. The test verification used regarding the accuracy of the creation of organisms included PCR and enzyme digestion and PCR reaction showed that a high percentage of the resulted clones have pGEM-SicA structure. Also, dual enzyme digestion for each of the two regions on the extracted plasmids indicated the presence of starting and ending sections of SicA gene with parts with lengths of 447 and 245 base pairs related to SicA gene in pGEM vector.

Salmonella are bacterial, gram-negative, anaerobic, moving, rod shaped. The genus Salmonella is divided into two species: Salmonella enterica and Salmonella bongori. Most species of Salmonella pathogens that result in disease in human belong to Salmonella enterica. The invading power of Salmonella is dependent on a 40kb area on the chromosome which

is located in Centisome area and is called Salmonella Pathogenecity Island. Kaniga *et al* (1995) studied the Homologues of Shigella invasive genes in Salmonella typhimurium culturing media that one of its results is mutation in SicA that prevents the secretion of sipB protein and results in the inability of the bacteria in invading cultured epithelial cells (Kaniga, 1995).

Tuchen *et al* (2000) studied SicA Complex in a Salmonella Typhimurium that indicated that elimination of SicA prevents from early bond between SipB and SipC (Tucker and Gala, 2000). In another study that was done by Surkhan, it was mentioned that the genes encoding chaperones are often adjacent to their target protein encoding gene and SicA chaperon directly encodes sipB in upstream (Sukhan, 2000). In a study in 2001 by Darwin and Miller on expression of pathogenicity genes of SicA and InvF in Salmonella typhimurium, it was shown that SicA protects from INVF destruction by proteolytic decomposition (Darwin and Miller, 2001). In a study by Ehrbar *et al* in 2003 on the role of INVB protein in type III secretion system, it was shown that SicA and SicP chaperons are encoded by SPI-1. Figueira *et al* in 2012 in a study on the function of second-N-terminal protein SipB showed that it is possible that sipB is not secreted through SPI-1 as it is not able to form a stable complex with SicA and sipc (Figueira and Holden, 2002) (Fig 1, 2).

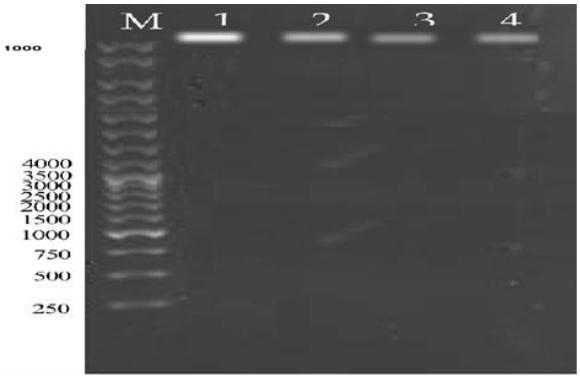

Fig. 1. Bond for the extraction of DNA from salmonella enteritidis.
M : 100bp marker manufacture fermentase, 4-1 :PCR products. 3-4-the production and proliferation sicA-up ,sicA-down and kan genes. sicA-up ,sicA-down and kan gene was amplified by the primers.bond length of the sicA-up gene is 447 bp and primer binding temperature is 61c.

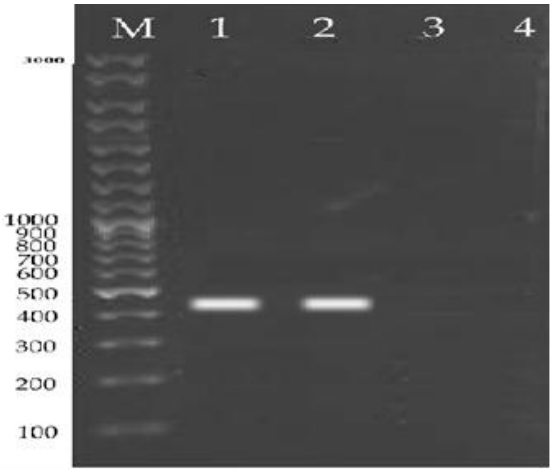

Fig. 2. 447 bp bond for sicA-up gene.
M : 100bp marker manufacture fermentase, 2-1 :PCR products sicA-up gene,3: negative control, Length bond for sicA-down is 245 bp and primer binding temperature is 58 c.

Conclusion

The results of the present study indicate the successful cloning of starting and ending encoder regions of SicA gene of Salmonella enteritidis in E. Coli bacteria. Thus, it seems that the structure produced in this study can be used as a gene vaccine candidate against Salmonella in future studies.

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