Escherichia coli with other food borne pathogens biology essay

Science, Biology

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Abstract

Pathogenic bacteria have the presence of virulence genes that encode for proteins which are responsible for their pathogenicity. Bacteria are known to acquire genes through horizontal gene transfer which also plays role in bacterial evolution. Genomic islands can excise themselves from the chromosomal DNA and can be transferred to the recipient. Pathogenicity islands have the presence of genes necessary for pathogenic mechanisms of bacteria. Intimin, an outer membrane protein coded by eae gene is present on a 35-kb pathogenicity island named Locus of Enterocyte Effacement (LEE). A clinical isolate of Escherichia coli was checked for the presence of the eae gene which was used as a marker for the pathogenicity island. To check for transfer of the pathogenicity island to Staphylococcus aureus and Salmonella, conjugation assay was performed using broth mating procedure. DNA was isolation was done followed by PCR amplification of the intimin gene. It was found that the gene did not transfer from E. coli to S. aureus, indicating that they are incompatible. Although the excision of the gene took place when E. coli was conjugated with Salmonella, the gene was not detected in Salmonella. This is probably because the gene did not integrate into the chromosomal DNA of Salmonella. Keywords: Pathogenicity island, Horizontal Gene Transfer, eae gene, intimin protein, conjugation.

Introduction

Albeit most of the Escherichia coli found in the human intestinal tract are non-pathogenic, there are some strains which are pathogenic and cause disease (Nataro and Kaper 1998). Pathogenic strains arise as a result of acquisition of virulence factors which may be encoded on mobile genetic elements like plasmids and bacteriophages, or distinct DNA segments, called pathogenicity islands, which can integrate themselves in the genomic DNA (Hacker et al. 1997). These genes can be acquired by Horizontal Gene Transfer mechanism. The HGT adds to the diversification of microorganisms. A significant part of the horizontal gene transfer is or has been facilitated by genomic islands (GEIs). Many GEI's have the capability to integrate into the genome of the host, excision, and transfer to a new host by transformation, conjugation or transduction (Juhas et al. 2009). One such genomic island present in the genomes of pathogenic bacteria known as Pathogenicity Island is responsible for their virulence factors (adhesins, toxins, invasins, protein secretion systems, iron uptake systems, and others). These islands were first described in Escherichia coli, but now their presence have been found in various other human, plant and animal pathogens (Hacker and Kaper 2000) These pathogenicity islands comprise up to 200 kb of DNA, with the G+C content differing from the rest of the genome (Hacker et al. 1997)Among many pathogenicity factors encoded by the E. coli genome, there exists the expression of intimin which is outer membrane protein which helps in the intimate attachment of the bacterial cells to the host. This intimate attachment of the bacteria with the host with the course of time damages the intestinal epithelium, disrupts the enteric environment and

causes diarrhea (Nataro and Kaper 1998)Intimin protein encoded by the eae gene is a part of a 35 kb pathogenicity island designated Locus of Enterocyte Effacement (LEE) (McDaniel et al. 1995). LEE also contains around 40 different proteins which include intimin receptor (Tir), a contact-dependent secretion system and a number of secreted products (Elliot et al. 1998). By type III secretion system intimin and intimin receptor are transferred to the host cell membrane during infection (Kaper et al. 1998). Genes of this pathogenicity island have a low GC content and an unusual codon usage pattern, suggesting that they are a foreign DNA that has spread in E. coli population. Therefore, there may be a probability that this segment of DNA can be transferred to other intestinal bacteria and make them pathogenic. In addition the eae gene contains 5'conserved region and 3' heterogeneous regions. Due to this observation, designing of universal PCR primers and allele –specific PCR primers was made possible. This led to the differentiation of 15 variants of eae gene, encoding 15 intimin types and subtypes (Blanco et al. 1999 and Blanco et al. 2004). Therefore, the present study was focused to see the possible exchange of the pathogenicity island between E. coli and other food-borne pathogens with eae gene as a marker.

MATERIALS AND METHODS

Chemicals: The chemicals and reagents used in the study were procured from HiMedia Laboratories, Mumbai and Sisco Research Laboratories, Mumbai.

Media composition:

Nutrient Agar: Peptic digest of animal tissue 5 g/l, sodium chloride 5 g/l, beef extract 1. 5 g/l, yeast extract 1. 5 g/l and agar 15 g/l. Brain Heart Infusion Agar: Calf brain 200 g/l, beef heart 250 g/l, proteose peptone 10 g/l, dextrose 2 g/l, sodium chloride 5g/l, disodium phosphate 2. 5 g/l and agar 15 g/l. Eosin Methylene Blue (EMB) agar: Peptic digest of animal tissue 10 g/l, dipotassium phosphate 2 g/l, lactose 5 g/l, sucrose 5 g/l, eosin - Y 0. 4 g/l, methylene blue 0. 065 g/l and agar 13. 500 g/l. Bacterial strains: Clinical isolate of E. coli, Staphylococcus aureus and Salmonella were obtained from MTCC, Chandigarh. Conjugation procedure: To determine the transferability of eae gene from E. coli to S. aureus and Salmonella, conjugation was carried out using broth mating procedure. Cultures of the donor and the recipient cell grown overnight in Brain Heart Infusion broth at 37°C were mixed with each other in a ratio of 1: 9 (donor : recipient) and incubated overnight at 37°C. Selection of transconjugants: Samples of this mixture were streaked on Eosin Methylene Blue (EMB) Agar to select for conjugated colonies. E. coli is differentiated from the recipient as it gives a green metallic sheen when grown on EMB agar. S. aureus and Salmonella colonies appear pink on EMB agar. The conjugants were selected and sub-cultured in nutrient agar slants. Genomic DNA isolation: CTAB method of genomic isolation was employed for extraction of genomic DNA from donor (E. coli), recipient (S. aureus and Salmonella) and transconjugants. The bacterial colonies were inoculated in 5 ml of Nutrient Agar broth by transferring a loopful culture from the plate culture and incubated at 37ºC in a shaker incubator. The liquid culture (1. 5 ml) were transferred to micro-centrifuge tubes and centrifuged at 8000 rpm

for 5 minutes. The supernatant was discarded and pellet was suspended in 567 µl of TE buffer by repeated pipetting. Then 10% SDS (30 µl) and proteinase K (3 µl) was added and mixed by inverting. The tubes were incubated at 37ºC in a water bath. 100 µl of 5M NaCl was added and mixed by inverting. 80 µl of CTAB/NaCl solution was added and mixed by inverting the tubes several times. The tubes were incubated at 65ºC in a water bath for 10 minutes. Equal volume of chloroform: isoamyl alcohol (24: 1) was added. The tubes were then centrifuged at 14000 rpm for 5 minutes at 4ºC. The aqueous layer was transferred to the fresh tubes. Equal volume of phenol: chloroform: isoamyl alcohol (25: 24: 1) was added to the aqueous layer. The tubes were centrifuged at 14000 rpm for 5 minutes at 4ºC. The aqueous layer was transferred to fresh tubes. Isopropanol (0. 6 volume) was added and the tubes were incubated for 30 minutes at -20ºC. The tubes were centrifuged at l2000 rpm for 5 minutes at 4ºC. The supernatant was removed and the pellet was washed with 70% ethanol (200 µl). The supernatant was removed and the pellet was air dried. The pellet was dissolved in 30 µl TE buffer and stored at -20ºC. PCR detection of eae gene: Gradient PCR was performed to optimize the annealing temperature of the gene. The forward and the reverse primers used for amplification of eae gene are as shown in Table 1. PCR reaction mixture was – water (6 µl), DNA (2 µl), forward primer (1 µl), reverse primer (1 µl), PCR master mix consisting of Taq Polymerase, MgCl2, dNTPs and tris buffer (10 µl).

Table 1: Forward and reverse primer for eae gene

Gene

Primer Sequence

Eae

Forward: ATGGCGCTAACCGATGTTAAReverse: CACTTTCTGAGCTGGCCTGAPCR conditions: An initial denaturation at 95°C for 2 min followed by further denaturation at 95°C for 1 min, annealing temperatures – a gradient of 47°C, 48°C, 49°C and 50°C, extension at 72°C for 1 min with 32 cycles of amplification and final extension at 72°C for 7 min. PCR was now performed for DNA isolated from all unconjugated and conjugated cultures at the optimum annealing temperature.

RESULTS AND DISCUSSION

Selection of transconjugants: Transconjugants were selected from the EMB plate on the basis of the difference in the colour of the colonies. E. coli colonies showed green metallic sheen. Salmonella appeared as purplish pink and S. aureus colonies appeared light pink in colour (Figure 1 and 2). Escherichia coliStaphylococcusC: UsersRAGAAppDataLocalMicrosoftWindowsTemporary Internet FilesContent. WordDSC_0817. jpg

Figure 1: Growth of S. aureus and E. coli on EMB agar

SalmonellaEscherichia coliC: UsersRAGADesktopDSC_0819. jpg

Figure 2: Growth of E. coli and Salmonella on EMB agar

Gradient PCR: Four different annealing temperatures used were - 47°C, 48°C, 49°C and 50°C. The PCR product was analyzed on 1% agarose gel. Further the amplification was visualized in gel documentation system as shown in Figure 3. Moreover, it was also interpreted that the size of the gene is approximately 1500 bp. 1 2 3 4 5C: UsersRAGADownloadscnjgtn 13313. JPG1500 bp

Figure 3: Gradient PCR amplification of eae gene from genomic DNA in E. coli

Lane 1 – 100 bp marker; lanes 2-5: 2 - 47°C, 3 – 48°C, 4 - 49°C, 5 - 50°C

PCR analysis: The amplification of the eae gene in unconjugated and conjugated cultures was performed (annealing temperature – 48ºC), the results of which are shown in Figure 4 and 5. 1 2 3 4 5C: UsersRAGADownloadsdhrmesh, conjugtn 29 march. JPG

Figure 4: PCR amplification of eae gene from genomic DNA of E. coli and Staphylococcus

Lane 1 – 100 bp ladder; lanes 2-5: 2 – Unconjugated E. coli, 3 – conjugated E. coli, 4 – unconjugated S. aureus, 5 – conjugated Staphylococcus

It is observed that eae gene is present in E. coli and is retained in E. coli after conjugation (Figure 4). The gene is absent in both unconjugated and conjugated S. aureus. It can thus, be inferred that the transfer of the gene did not take place from E. coli to S. aureus through horizontal gene transfer. This indicated that the two bacteria are incompatible with each other. A non-

specific amplified band too was observed. This can arise either due to presence of another copy of the gene at different position in the genome or a non-specific amplification. 1 2 3 4 5C: UsersRAGADownloadsdhrmesh, conjugtn 29 march. JPG

Figure 5: PCR amplification of eae gene from genomic DNA of E. coli and Salmonella

Lane 1 – 100 bp ladder; lanes 2-5: 2 – Unconjugated E. coli, 3 – conjugated E. coli, 4 – unconjugated Salmonella, 5 – conjugated Salmonella

It is observed that the amplification of the eae gene takes place only in unconjugated E. coli and is absent in conjugated E. coli (Fig 5). This indicates the loss of the gene from E. coli on conjugation with Salmonella. However, the gene is also absent in both unconjugated and conjugated Salmonella. It can be stated that although the gene was excised from E. coli stable integration of the gene did not take place in Salmonella. Stable uptake of the DNA depends on the molecular factors governing the integration of DNA into the recipient’s chromosome and the nutrient conditions. Bacterial genome codes for core genes that code for essential metabolic functions. Besides this their genomes also have a number of other additional genes which are beneficial in certain environmental conditions, these genes are attained by horizontal gene transfer.