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MOLECULAR CHARACTERIZATION OF FOOD BORNE PATHOGENS FOR *E.coli* AND *Staphylococcus aureus* BY USING PCR METHOD

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ABSTRACT

Molecular characterization is used to characterize the organism at the molecular level without any effect of environment or development or physiological state of the organism. Molecular characterization is done using Primers they are fragments of DNA that can be identified with the whole genome. The main goal of this project is to characterize food borne pathogen such as ***Staphylococcus aureus*** which is an opportunistic pathogen and *Escherichia coli* present in milk which is a widely consumed food product These pathogens are associated in causing human diseases. These pathogens can also be found collecting and isolating the samples to find its morphology accordingly but it requires huge time and also in a morphological note there are many similar characteristics for the organisms so it is difficult to identify and characterize So, the characterization is done using primers in PCR (Polymerase Chain Reaction). This method takes very less time and also we can share the DNA fingerprinting data to the public health agencies regarding consuming of the particular product.

Key Words: Molecular characterization, PCR, Human Diseases, *Staphylococcus aureus, Escherichia coli,Primers*

1.INTRODUCTION

In the earlier days there was a wide spread of diseases the cause of disease was unknown and later it was found that the diseases was due to the food borne pathogens so as a result food agencies took an effort to qualify the consumables in a rapid manner instead of taking huge amount of time so, as a result Molecular characterization came into action instead of collecting, isolating and analyzing the sample and qualifying it after conforming the absence of pathogen Molecular characterization method was adopted so that that analysis is done in a faster manner and therefore the spread of diseases can be considerably reduced.

In this project the sample which is taken for characterizing the pathogens is milk the milk sample is collected from different cow farms so that it gives desirable strains of organisms generally while drawing milk from cows udder due to improper sanitization procedure like washing of udder before drawing milk and also the person who is drawing milk etc. There is a chance of contamination of bacterial organisms which in turn causes diseases**.** Just like all people carry microbes, all animals do as well. Sometimes the microbes that cows carry can be a problem.

"commensal organisms" that exist within cow itself (without causing any diseases) but it serves as a human pathogen .There is a chance that this organism causing diseases in human beings when the milk is consumed.some of the organisms which are present in milk are Micrococci, Bacilli, Staphylococci, Lactobacilli, Pseudomonas, and coliforms *Escherichia coli and Staphylococcus aureus* are some of the opportunistic pathogens.

2.MATERIALS AND METHODS

Milk Sample Collection

Milk sample is collected from two different cow farms for desired strains of bacterial Organism.The milk sample is collected in cow farm house located in Madhavaram,Chennai.

DNA Isolation

DNA isolation involves three major steps they are,Cell lysis DNA ,Separation Of DNA from Protein and other Cell lysis,Precipitation of DNA.

Cell Lysis

The overnight Milk sample will be washed by salt solution I &II, which chelates the metal ions from the cells, thus making the cell wall susceptible to breakage.Mixingthecellswith detergents like SDS (sodium dodecyl sulfate) will break down the cell walls (if present) and cell membranes

A feed sample (0.5) was taken and frozen in a pestle and mortar at 20 8C and was ground to a fine powder and suspended in TE buffer 5 ml). This was centrifuged and the supernatant was collected. The DNA was isolated using the procedure described above after treatment with proteinase K and CTAB solution, and finally precipitated with 2-propanol.The isolated DNA was checked for its purity by gel electrophoresis and used for PCR. Similarly DNA was isolated from different food samples and used for detecting the pathogenic bacteria by PCR.

Preparation of TBE 10X Buffer

0.5X TEB by dissolving one volume of 10X TEB buffer and add 19 volumes of double distilled water. This gives 0.5XTEB running buffer.

Spin column chromatography

The milk sample was loaded in the spin column, the stationary phase Consists of silica selective binding of nucleic acid to a silica membrane To the extracted DNA sample washing buffer 1 was added and then it was centrifuged at 400 rpm for 4 mins to remove the debris particles and then the supernatant was discarded and then the pellet was again treated with wash buffer 2 and centrifuged at 400 rpm for 4 mins then the supernatant was discarded and the extracted nucleic acid sample was treated with 70% ethanol and centrifuged and then the sample was run in agarose gel electrophoresis tank in order for DNA band Visualization 0.380 mg of agarose was dissolved in DNA buffer and heated in a heating mantle. At 100ºC after heating crystal clear solution was formed It was then allowed to cool for a bearable temperature and then it was poured on to the tank and then gel loading dyes are added to the wells 100 V was set and then the sample is allowed to move from negative to positive terminal and then it was left for about 30 mins for the sample to move Then the gel was taken and viewed under the UV trans illuminator. The DNA sample was subjected to PCR using primers.

Fig 1: Spin Column

Procedure for PCR

In PCR, the temperature of the reaction is cycled between three different levels. The first level is at a high temperature and is used to break the hydrogen bond between complementary bases that hold the two strands of the template together. This step is called denaturation. The reaction is taken down to a lower temperature at which point the two PCR primers can anneal to the template strands. This step is called annealing. In a final step called extension, the reaction is brought up to an intermediate temperature at which the DNA polymerase adds nucleotide onto the ends of the annealed primers.The steps of denaturation, annealing, and extension make up one complete cycle.Making enough copy of DNA for analysis by gel electrophoresis typically requires from 25 to 40 cycles

PCR Cycling parameters

Temperature 1: Initial denaturation at 95□C for 5 minutes

Temperature 2: Denaturation at 95□C for 1 minute

Temperature 3: Annealing at 58□C for 1 minute

Temperature 4: Extension at 72□C for 1 minute

Repeat – Go to step 2 for 25 cycles

Temperature 5: Final extension at 72□C for 1minute

Temperature 6: Hold 4°C

Components Mix Volume for PCR

Sterile Water,Master Mix(10X Tag polymerase buffer 10mM dNtp mix,Tag Polymerase),Primer (Forward and Reverse),Template DNA Totally 20.0 µl of Mix volume.

Fig 2: PCR Machine

Design Primers for *E.coli*

For *E. coli* the primer sequence was based on the gene sequence of afa. This gene is responsible for pathogenicity and is specific to E. coli. The primer sequence for the amplification of the afa gene from E. coli is: forward primer, 5^1 GCT GGG CAG CAA ACT GAT AAC TCT C 3^1; reverse primer, 5^1 CAT CAA GCT GTT TGT TCG TCC GCC G 3^1.

3.RESULTS

DNA was isolated from different micro-organisms and the purity and integrity of the isolated DNA was examined by agarose gel electrophoresis. A high-molecular-mass band with minimum shearing was observed. DNA isolated from different organisms such as *Staphylococcus*, *E. coli* and unknown bacteria isolated from feed and food samples also exhibited the same property. Using specific primers PCR was carried out on DNA isolated from different micro-organisms.The conditions of PCR were carefully Standardized and all the parameters were established. The optimum annealing temperature was found to be 56.8C and within 20 cycles a substantial band was amplified only from Staphylococcus strains and not from non-Staphylococcus strains. The size of the amplified product was 120 bp as shown by comparison with marker DNA method for the rapid identification of *Staphylococcus* was also standardized. In this procedure DNA was isolated from a single colony.
PCR was conducted to detect the specific DNA.

The agarose gel electrophoresis pattern of the PCR products showed that DNA from all the Staphylococcus colonies was amplified with the specific sets of primers, but under these PCR conditions DNA from non- Staphylococcus colonies was not amplified, and also no non-specific products were amplified. PCR amplification was found only in *Staphylococcus* strains and not in non-*staphylococcus* strains regardless of the method of template preparation. Afterwards, analysis of PCR products by gel electrophoresis requires clearly different sizes of amplified fragments. The name, sequences of the primers, melting temperature, and fragment sizes PCR test is based on species-specific sequences of the thermo nuclease gene and includes an internal positive control that targets a highly conserved region of 16S rDNA as a check on the desired course of the PCR. This test could be used readily in routine laboratory procedures for epidemiological studies and to evaluate the true frequency of *S. aureus* in food. In a first approach, two different DNA isolation methodsweredirectlyappliedtocontaminatedmilks ampleswithouttheenrichment step. Neither the *Genomic DNA From Bacteria in Milk* kit, nor the MCH was able to reliably detect bacterial presence below the contamination level of 10^4 CFU/ml (data not showed).

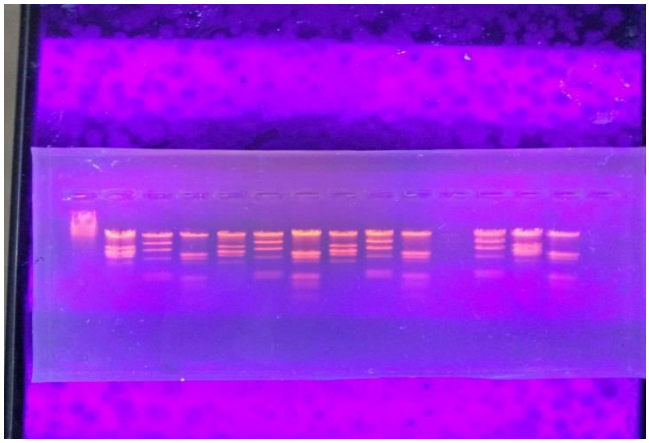

Fig.3 *PCR results* of E.coli *and S.aureus*

4.DISCUSSION

The DNA was isolated from different micro-organisms and the purity and integrity of the isolated DNA was examined by agarose gel electrophoresis. A high-molecular-mass band with minimum shearing was observed. DNA isolated from different organisms such as *Staphylococcus*, *E. coli* and unknown bacteria isolated from feed and food samples also exhibited the same property Using specific primers PCR was carried out on DNA isolated from different micro-organisms. The optimum annealing temperature was found to be 56.8C and within 20 cycles a substantial band was amplified only from Staphylococcus strains

and not from non-Staphylococcus strains. The five sample units revealed the contamination. Moreover, a perfect agreement was observed in results obtained through the two Real-Time multiplex PCR In this study a multiple platform of simultaneous detection of *E.coli and S.aureus*in milk was developed the recovery of bacteria from foods, including milk, is often complicated by the very low level of contamination. For this reason, the selection of a proper enrichment medium can improve the sensitivity and reproducibility of the test. Some broths for multi- pathogen enrichment are either commercially available as is Universal PR enrichment Broth(UPB),or have been experimentally proved.

5. CONCLUSIONS

The milk sample was taken for analysis for the purpose of detection of bacterial pathogens which causes or responsible for causing human diseases the target pathogen in this study is *staphlycoccusaureus* and *Escherchia coli* which is generally present in the feed samples such as milk The main aim is to detect the presence of pathogen in a quicker method rather than using classical methods The rapid method which was adopted was PCR method using Polymease chain reaction it can be detected easily with the help of primer complement to the pathogenic strain . Column chromatography method was used to extract the DNA from the bacterial organism present in the milk and therefore spread of diseases can be prevented as if we qualify the food or milk in a faster manner and therefore the report can be generated to food council also reporting regarding the adulteration of food or milk sample In addition it also helps to examine whether the cattle is contaminated.

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