# MOLECULAR CHARACTERIZATION OF FOOD BORNE PATHOGENS 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.*

**Keywords:** Primer, PCR, Human Diseases, *Staphylococcus aureus, Escherichia coli.*

## 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 [1].

Dairy processing facilities have many routes for the entry of contaminating microbes. First, as a nutrient-rich liquid, milk provides an ideal environment for microbial growth. Second, dairy processing plants are full of areas where “foot traffic” from employees can be accompanied by microbes [2]. Some dairy cows spend much of their time grazing in pastures, where they come in contact with a variety of environmental microbes. In other cases, cows are confined to buildings, wherein more crowded conditions the bacteria can grow and spread from cow to cow.

*Escherichia coli* have been associated with a number of food-borne outbreaks and is often a cause of bloody diarrhea (hemorrhagic colitis.) Frequently associated with dairy cattle, microbial contamination of raw milk and soft cheeses can result in disease. *Staphyloccous aureus* produces a toxin that causes explosive vomiting and is a common "potluck" cause of food poisoning. Food poisoning from *Staphyloccous aureus* is not caused by an infection with the bacteria, but rather the bacteria release toxins into food which is left out at room temperature. Upon heating, the bacteria are killed, but the toxin, retains. Staphylococci (CNS) are opportunistic pathogens that are currently emerging as causative agents of human disease. To devise a rapid and accurate identification method, *sodA*-specific primers were designed to demonstrate that species-specific multiplex polymerase chain reaction (PCR) can be used for the identification of CNS species. The accuracy of this method was higher than that of phenotypic identification; the method is simple and less time-consuming than 16S rRNA [3].

## 2. Materials and Methods

Milk sample is collected from two different cow farms for desired strains of bacterial Organism. The milk sample was collected in cow farm house located in Madhavaram Chennai-600118. 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. Mixing the cells with detergents like SDS (sodium dodecyl sulfate) will break down the cell walls (if present) and cell membranes Lysozyme may be added to bacterial cells as an aid for lysis and to remove protein molecules from the DNA. The addition of salt solution will precipitate the proteins; as a result the genomic DNA can be separated after centrifugation. RNase has been added to remove RNA. The addition of salt solution will precipitate the proteins; as a result the genomic DNA can be separated after centrifugation. RNase has been added to remove RNA

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.

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, 5¹ GCT GGG CAG CAA ACT GAT AAC TCT C 3¹; reverse primer, 5¹ CAT CAA GCT GTT TGT TCG TCC GCC G 3¹. For each run, the whole PCR mix without DNA template was used as a negative control. The amplification reactions were carried outAfter amplification, 10 ml of reaction mixture was analyzed by electrophoresis on a 1.2% agarose gel in Tris-Borate-EDTA buffer (89 mMTris, 89 mM boric acid, 2 mM EDTA) at 80 V for 45 min and stained by Ethidium bromide. Presence or absence and size of the PCR products were checked.

## 3. Results and Discussions

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 [4]. Using specific primers PCR was carried out on DNA isolated from different micro-organisms. The conditions of the 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. Thus a simple and rapid technique was developed for the identification of *staphylococcus* [5].

Wealth of data allows alignments of a given gene originating from several strains in order to check sequence conservation or divergence nuc gene of *S. aureus* (SAnuc) has already been used to design primers for a simple PCR test aiming at the identification of *S. aureus* strains To get a positive control on the desired course of the PCR, the highly conserved 16S ribosomal RNA gene sequence of *S. aureus* and was aligned and a common sequence was chosen A primer pair was then designed (16S1, 16S2) that annealed to the 16S ribosomal RNA gene of both species. The multiplex PCR requires that primers share the same melting temperature (Tm), with no possibility of cross-hybridization. Afterwards, analysis of PCR products by gel electrophoresis requires clearly different sizes of amplified fragments [6-7]. 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 [8]. In a first approach, two different DNA isolation methods were directly applied to contaminated milk samples without the enrichment 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). After introduction of the enrichment step with the optimized medium, the sensitivity obtained by both DNA isolation systems (*Genomic DNA From Bacteria* and MCH) rose to a very high level, corresponding to 1CFU of each pathogen in 125 ml milk divided into five a aliquots for an over view of the entire experiment). Considered in their totality, 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.

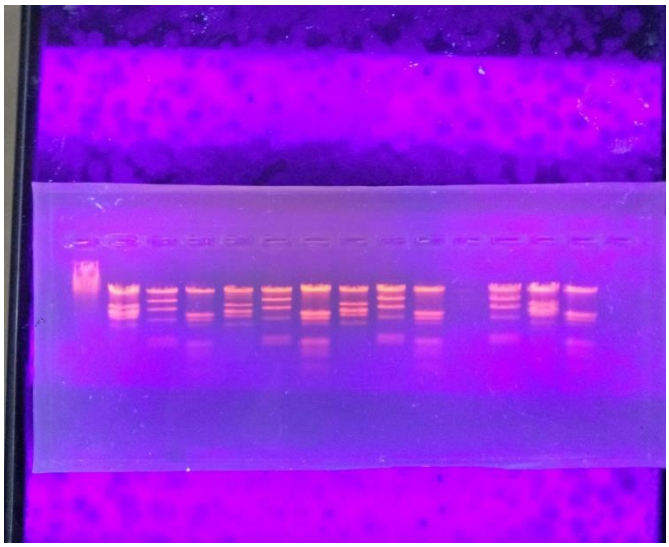

**Fig 1.** PCR results *of E.coli* and *S.aureus*

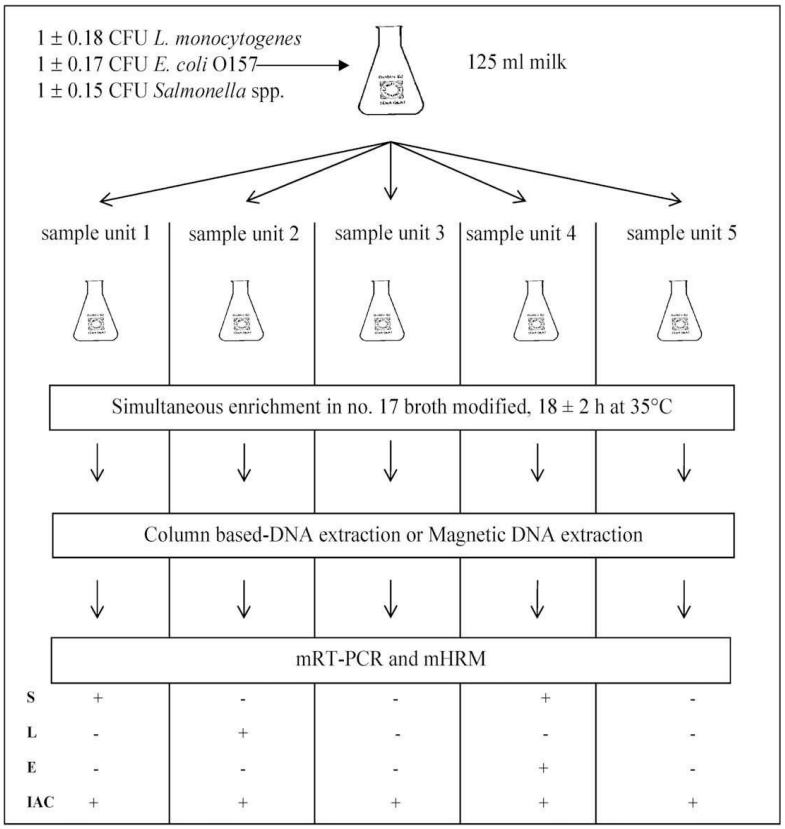


**Fig 2.** Sample runes in PCR

## 4. Conclusion

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 *staphlycoccus aureus* 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 Polymerase 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.

## 5. References

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