

Research Article

Establishment of a Rapid Polymerase Chain Reaction Method for the Detection of *Staphylococcus aureus* in Food

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Abstract: With the improvement of living standards and frequently occurrence of food safety incidents, People pay more and more attention to food safety. *Staphylococcus aureus* is one of pathogens which could cause food spoilage. In this study, specifically primers has been designed and a rapid method has been established to detect *S. aureus* in food to shorten time of detection, increase the sensitivity of method and provide a technical help. PCR method has higher specificity, sensitivity, quickness and convenience, which may provide a new approach to detect SA in food quickly. *Staphylococcus aureus* was detected by PCR samples from food poisoning, in the case of the exclusion of other pathogens that can prompt this food poisoning is caused by *Staphylococcus aureus*. *S. aureus* enterotoxin is the main cause of food poisoning, which can be identified on its type enterotoxin produced under conditions permitting.

Keywords: Food, rapid detection system, *Staphylococcus aureus*

INTRODUCTION

According to *Staphylococcus aureus* is a genus belonging to Gram-positive bacteria. Diameter of 0.8~1.5 μm , *Staphylococcus aureus* without flagella, spores, with a small part of the capsule, can't be moved. Optimum growth temperature is 37°C, PH value is 7.4. *Staphylococcus aureus* is highly salt tolerance, growth can be good in high salt medium. *S. aureus* can also break down lactose, maltose, sucrose, ribose, turnsole, mannose acid no gas. It can be broken down mannitol and produce hemolytic substances, so training will produce hemolytic on blood agar. Plasma clotting enzyme can be produced by generating plasma coagulation, which is different from the main properties of the other *Staphylococcus aureus*.

S. aureus can produce a variety of toxins, *Staphylococcus aureus*, which is the cause of food poisoning, the toxin secreted by serology, including 14 kinds Some toxins can cause damage to human leukocytes and macrophages, there is can lead to fibrin deposition in the blood, which can lead to human treatment. In addition, purulent infection pathogens in humans, *Staphylococcus aureus* is the most common pathogen. *Staphylococcus aureus* can even cause human purulent infections, pneumonia, pericarditis and other diseases (Kumar and Mittal, 2010).

In this study, *Staphylococcus aureus* was detected by PCR samples from food poisoning, in the case of the exclusion of other pathogens that can prompt this food poisoning is caused by *Staphylococcus aureus*. *S. aureus* enterotoxin is the main cause of food

poisoning, which can be identified on its type enterotoxin produced under conditions permitting.

MATERIALS AND METHODS

Pathogenicity of *S. aureus*: *Staphylococcus aureus* is the most common human purulent infection of pathogens that can cause localized purulent infection, can also cause pneumonia, pseudo membranous colitis, pericarditis and even sepsis and other systemic infections. *Staphylococcus aureus*-induced disease force depends on how toxins and invasive enzyme which produces: hemolytic toxins: toxin can damage the platelet destruction lysosomes, causing the body ischemia and necrosis; leucocidin can destroy people's white blood cells and macrophages: coagulase: When *S. aureus* invades the human body, the enzyme in the blood or plasma fibrin deposition on the cell surface or coagulation, hinder phagocytic role in the formation of staphylococcal infections easily localized with this enzyme related; DNase: *Staphylococcus aureus* DNase can withstand high temperatures, can be used as basis for the identification of *Staphylococcus aureus* (Atural News, 2008).

Enterotoxin: *Staphylococcus aureus* can produce several kinds of acute gastroenteritis caused by enterotoxin protein, total A, B, C (C1, C2 C3.), D, E, G, H, 1, J, K, L, M, N 14 serotypes. Outbreak of food poisoning is the most common serotypes A and D, B and C, followed, in which the most virulent type. It is reported that excessive amounts of *Staphylococcus aureus* can cause food poisoning symptoms of an

effective amount to produce enterotoxin. When *Staphylococcus aureus* containing starch and water pollution more food, such as meat and meat products, milk and dairy products, eggs, etc., when the temperature conditions are suitable, after 8-10 h to generate a considerable amount of enterotoxin. One hundred degree Celsius enterotoxin can withstand without being damaged by boiling 30 min. Therefore, the destruction of the food is in *S. aureus* at 100°C heating food 2 h. Enterotoxin can be stimulated intestinal epithelial cells by cyclophosphamide and cyclic adenosine phosphate birds past the secretary pathway and two independent cyclic nucleotide result in diarrhea in sodium channels, water secretion. While studies have shown that *S. aureus* cells on small intestinal no direct role in the destruction and to the complete molecule absorbed into the bloodstream through the digestive tract, after reaching the central nervous system to stimulate the vomiting center and vomiting as a result of the main symptoms of food poisoning (Brooks, 1986).

Epidemiology of *Staphylococcus aureus* generally have the following characteristics: seasonal distribution, more common in spring and summer: more kinds of food poisoning, mainly in all kinds of cooked meat products, milk and dairy products, eggs and egg products, followed by dairy products containing frozen food poisoning incidents caused by individual starchy foods have also been reported. Food sources of *Staphylococcus aureus* and enterotoxin formation conditions: *Staphylococcus aureus* ubiquitous in nature, humans and animals nose, throat, digestive tract colonization were high, healthy people carrier was a 30 to 20%, upper respiratory tract infection nasal infection rate 83%, so the site of infection to humans and animals often become purulent sources "In general, *Staphylococcus aureus* can contaminate food through the following channels: staff cook food processing or sales personnel carrier, resulting in food contamination; before processing itself contaminated food or contaminated during processing, resulting in intestinal toxins, causing food poisoning; cooked food products packaging lax contamination during transport; cows suffering from mastitis or livestock local pyogenic purulent, the contamination of other parts of the body.

***Staphylococcus aureus* prevent contamination of food, mainly from the following aspects Note:** To prevent contamination of various foods contaminated crowd: the production and processing of personnel regularly conduct health checks, suffering from localized pyogenic infections (such as scabies, fingers purulent, etc.), upper respiratory tract infection (such as sinusitis, pyogenic pneumonia, oral diseases, etc.) who want to temporarily stop their work or replacement jobs. Prevent *Staphylococcus aureus* contamination of milk and its products: milk factory to check on a regular

basis, such as cow's breast, not crowded with suffering from pyogenic mastitis milk; milk after extrusion, to quickly cool to 10e below and prevent toxins generated, bacterial growth of the dairy milk as raw material to be sterilized, attention cryopreservation. Suffering local purulent infection of poultry, livestock carcasses of sick animals should, sick poultry processing, after the removal of the lesion, according to conditions carnivorous high temperature treatment to cooked products sold.

Rapid detection method: In recent years, with the improvement of people's living standards, food safety requirements are high, especially due to food poisoning caused by pathogenic organisms, has aroused more and more attention, traditional detection methods to detect the cause of food tedious and time-consuming step of germs, subject to enrichment culture, isolation and culture, morphological observation, physiological and biochemical reactions serology and the necessary animal testing and other processes and traditional methods can't be difficult for those cultured microbial detection and PCR technique, just a few hours, can be detected by electrophoresis 0.1 mg DNA contains only a few copies of the template sequence; bacteria by PCR amplification conservative DNA fragments, but also for those who can't be cultured in artificial microbial detection (Feng *et al.*, 2008).

PCR has the following characteristics: As in base pairing with the template DNA primers to correct specific binding, DNA polymerase synthesis reaction fidelity and specificity conserved target genes together determine the PCR of the high specificity; PCR has a high sensitivity, because the amount of PCR products is increasing exponentially, Pique can amplify the magnitude of the initial test template to the microgram level. From one million cells were detected in a target cell; bacterial detection, the minimum detection rate of up to three bacteria; simple, fast, through the use of high temperature Taq DNA polymerase, disposable After the reaction solution was added, DNA amplification for the denaturation-annealing-extension reactions, usually in 2 to 4 h to complete the amplification reaction, the amplification product is easy to analyze, necessarily using radionuclides, no radioactive contamination, easy to promote, purity requirements of the specimen low, wide adaptation.

CPR techniques because of specificity, high sensitivity, fast and accurate, high degree of automation in the medical life sciences has been widely used. In recent years, with the improvement of people's living standards, food safety requirements are high, especially due to food poisoning caused by pathogenic organisms, has aroused more and more attention, traditional detection methods to detect the cause of food tedious and time-consuming step of germs, subject to enrichment culture, isolation and culture,

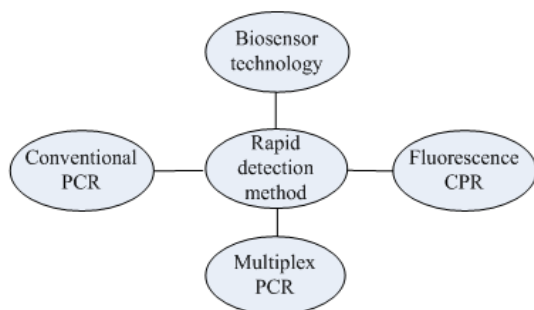


Fig. 1: The rapid detection method

morphological observation, physiological and biochemical reactions serology and the necessary animal testing and other processes and traditional methods can't be difficult for those cultured microbial detection "and PCR technique, only a few hours, can be detected by electrophoresis 0.1 mg DNA contains only a few copies of the template sequence; bacteria by PCR amplification of conserved DNA fragments of those microorganisms may be detected by a human can't be cultured.

PCR method provides the possibility of copying the natural CPR simulation of DNA fragments for the detection of specific genes by DNA polymerase gene is amplified, the primer complementary to the template DNA combined with a later role in the DNA polymerase according to the principle of base pairing, from the beginning the synthesis of DNA primers complementary to the template DNA strand, denatured annealing, extension and other one cycle, doubling the number of DNA strands, Since then people will apply this technology to different areas, has made tremendous achievements PCR technology inspection of medical outcomes taken particularly prominent (Francois *et al.*, 2008). Figure 1 shows the rapid detection method.

Multiplex PCR: Using multiple sets of primers in a single reaction tube, a process for the amplification of a plurality of different regions of the same DNA template or templates is multiplex PCR, can meet the needs of simultaneous analysis of different DNA sequences. Traditional CPR method for detecting the toxin gene PCR reactions need to be 1760 times, but with this multiplex PCR method to detect only need 352 PCR reactions can greatly save time and money, Multiplex CPR system detects *Staphylococcus aureus* toxin genes, with sensitive, specificity, fast, easy to repeat, low cost, etc., which has been successfully used in food detection of *Staphylococcus aureus*.

CPR fluorescence method, U.S. PE (PerkinElmer) company developed a fluorescent PCR technology, not only to achieve a PCR from qualitative to quantitative leap and compared with conventional CPR, specificity stronger, High degree of automation and can effectively solve the problem of PCR contamination, which has

been more widely used, fluorescence CPR technology into the reaction system in an ordinary fluorescent group, a detection process using the accumulation of the fluorescence signal of the PCR reaction and finally the standard curve for quantification of the unknown template. due to the highly specific biosensors and bioreactors signals specific recognition of biologically active substances produced by highly amplified due to sensitivity together, so it has fast speed, low-cost, test sample trace, the biological function of the film can be repeated many times use and other characteristics, can be used to detect many substances. The rapid detection system for better applied to the detection of different types of food in *Staphylococcus aureus*.

PCR method compared with other methods for rapid detection: In recent years, the development and application of up higher accuracy and rapid detection methods have their own characteristics, the subject of the use of PCR pathogenic *Staphylococcus aureus* medium direct detection method and two kinds of food in the rapid detection of *Staphylococcus aureus* and GB4789.10 a 94 methods were compared to determine the direct detection of specific dairy products in the PRC *Staphylococcus aureus* approach, in line with the rate sensitivity. *Staphylococcus aureus* routine testing procedures: sample enrichment, separation, sub-pure, Gram staining/nuclease resistant test/coagulase test/biochemical reaction test/inspection enterotoxin. Three to five days whole detection process is completed, the detection time is long, the detection procedures are complicated, so scientists according enterotoxin and heat which produces a number of other nucleic acid enzymes developed rapid detection methods.

For *Staphylococcus aureus* enterotoxin detection, in the past mainly by immunological methods to detect, but often false positive or false negative results because some strains, although it contains the toxin gene, but the gene expression of bad or lack of stable genetic component the false-negative results; serotype strains prone to some cross-reactivity and often false positive. With the application and development of PCR technology, the diagnostic detection of *Staphylococcus aureus* enterotoxin has been improved.

Both used for rapid detection of pathogenic *Staphylococcus aureus* medium for: Petrifilm RSA Count Plate (USA 3M Company to develop and produce), is a rapid detection count flat film type *Staphylococcus aureus*, Baird a parker ten (*Staphylococcus aureus* detection French Merieux development and production of Baird a parker rabbit plasma fibrinogen agar plus count tablet) RPFA gar principle: Petrifilm RSA test film is composed of two parts: the first part is a medium piece by *Staphylococcus aureus*, Baird parker this medium

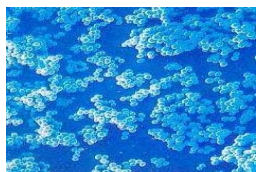


Fig. 2: The *Staphylococcus aureus*

containing an amended nutrients coupled with cold water soluble gum. The second part is a heat-resistant nuclease (Tnas) e reaction tablets containing DNA and toluidine blue and four wow Om indicator, this indicator helps the colony count and determine the presence of heat-resistant staphylococcal nuclease (Sievert *et al.*, 2008).

In the air, water, soil and human and animal excrement in nature there are a wide range of *Staphylococcus aureus* in healthy human nasal cavity, throat, also found on the hair, so if food production and marketing chain in production due to who are likely to result in improper operation *Staphylococcus aureus* contamination. Since the spring and summer temperatures and humidity are high, *Staphylococcus aureus* in this environment can multiply easily lead to the seasonal distribution of *Staphylococcus aureus*. Susceptible *Staphylococcus aureus* have contamination of a wide variety of foods, including meat products, eggs and dairy products, food-based.

Petrifilm RSA detection sheet, heat must be used with Petrifilm nuclease reaction pad, colonies will not be displayed alone as an auxiliary indicator of colonies counted in the reaction plate, but not in the detection sheet Petrifilm RSA. Baird a Parke+rRPF Agar, this medium is rich in nutrients, which buried instead of Asia hoof potassium chloride, so that the colony color was black, RFP and cattle supplemented with rabbit plasma fibrinogen to detect coagulase vitality, trypsin can suppress all or part of coagulase-positive colonies around the surrounding halo precipitate dissolved fibrin and therefore where the existence of coagulase-positive *Staphylococcus aureus*, will present the halo ring black colonies in the medium, to confirm and for counting (Atural, 2008). Figure 2 shows the *Staphylococcus aureus*.

Establishment of PCR detection methods: Given the dangers of *S. aureus* itself and cause serious food poisoning cases, the establishment of methods for rapid detection of *Staphylococcus aureus* from food is very necessary. Overseas food has been established to detect *Staphylococcus aureus* Polymerase Chain Reaction (PCR) method, but this method is not yet applied to the food it was detected in the past. This study focused on the characteristics of pathogenic *Staphylococcus aureus*, select specific primers, established PCR method for rapid detection of *Staphylococcus aureus* in food. In order to shorten the detection time of food poisoning caused by *Staphylococcus aureus*, improve the sensitivity of detection method and provide technical

support for the rapid diagnosis of *S. aureus* cause foodborne illness.

Staphylococcus aureus pure bacterial DNA extraction DNA was extracted by pure bacterial cells FrA filter method: *Staphylococcus aureus* access nutrient broth medium and cultured with shaking overnight and then diluted with distilled water to make *Staphylococcus aureus* concentrations were 100 cfu/mL *10, 2 cfu/mL, Lin L with 20 points to FTA filter, 56e and dried for 15 to 20 and n, then washed 2 times with FTA buffer, 56e dry "reaction can be used as a template CPR. *S. aureus* DNA was extracted for PCR amplification to detect specific primers. FTA filter the extracted DNA as a template, PCR reaction was performed to determine the sensitivity of the PCR reaction.

DNA extracted from *Staphylococcus aureus* as a template, the CPR has been established to detect reaction procedure. The product mix LPR 5 taken on a 2% agarose gel electrophoresis, using a gel imaging system observation and imaging. *Staphylococcus aureus* in food flotation step DNA extraction method: Take 10 nL homogenate to 1000 xg centrifuge 10 min. Supernatant join another sterile centrifuge tube to 1400x0 g, centrifugal 10 min, precipitated with 500 L of saline suspension worship, join 0.025 times the volume of ethyl acetate cool, mix two oscillators and n, then 17000 xg, centrifugal 10 min the supernatant was removed, mixed with 20 L of normal saline precipitate was suspended by adding the diameter of 0.200~FTA membrane sheet 56e and then dried, the dried membrane sheet TFA, 10% SDS solution was added to 200 L of boiling 10 and thanks to n, washed with FTA dedicated buffer twice and then washed twice with TE buffer, after drying, the reaction can be used as a template CPR.

Take 10 mL homogenates at 1000 g precipitated with 500 L of saline suspension worship, join 0.025 times the volume of ethyl acetate cool, shaker and mix 2 and n, then 17000 xg, centrifuged 10 min to remove the supernatant was precipitated with 300 mixed L1011MTE (pH 7.8) to dissolve. Thanks to the 5 L (10 m aged mL), was added to the lysostaphin liquid, 37e incubated 1 h, during continuous vigorous shaking, fight L10% was added to the liquid, boiled 5 min. An equal volume of chloroform was added to the mixture and mixing thoroughly shaken, 17000 xg centrifuge 10L discard the pellet, the supernatant retained. The supernatant was transferred to a new centrifuge tube and 0.1 volume of 0.25 M ammonium acetate (pH 5.4). 25 volume of cold ethanol and 5 uL (10 mL) glycogen precipitated DNA, the mixture 17000 Xg, centrifuged 20 after min DNA precipitated dry spell with a 100 L sterile distilled water to dissolve, the other to take a spare artificially contaminated samples homogenized 37e enrichment 18 h, according to the above steps *Staphylococcus aureus* DNA extracted directly.

The CPR plays important roles in rapid detection, but it is often difficult to get enough samples from

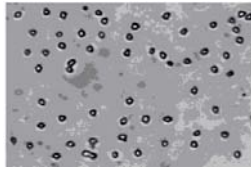


Fig. 3: The cell image of *Staphylococcus aureus*

unknown environment. Therefore, trial-and error unsupervised learning is more applicable for rapid detection. The detection of *Staphylococcus aureus* preliminary study, the detection time can be controlled in a short time, high accuracy and low cost of detection. As in the previous process can be secreted by *S. aureus* using the specific combination of the enzyme and the specific stain for staining cells, which can continue to shorten the incubation time of *S. aureus*, *Staphylococcus aureus* and thus make detection time is further shortened. And because of the diversity of the food, its texture is different and therefore need different types of food pre-treatment methods and classification to discuss and refine the test to verify and establish different types of food in the detection of *Staphylococcus aureus* pre-treatment method, a standardized pre-processing step. The rapid detection system for better applied to the detection of different types of food in *Staphylococcus aureus* (Takano *et al.*, 2007).

The image processing and recognition of cells:

Through the image preprocessing, segmentation, morphological processing and post-edge extraction, image of tiny particles and other impurities have been basically eliminated. This section of the measure can eliminate bacteria and bacterial contaminant particles by size equivalent. Through the bacterial form of the bacteria in different aspects of the characteristic parameters, computer pattern recognition the selection and extraction of the digital recording. Because the image acquisition process by the interference of external factors, so the need for an image obtained by the image acquisition processing and analysis to have in order to achieve the purpose of the computer can accurately identify the target object.

This chapter of the image pre-processing, image segmentation, feature recognition and image of the target object from, while the characteristic value of the target object extraction, classification and recognition to achieve the target object by means of artificial neural

networks. Figure 3 shows the Cell image of *Staphylococcus aureus*.

Since the cell edge of the divided image has been many burrs along the breakpoint in the background, there are some foreign particles due to the presence of these defects lead to inaccurate edge tracking cells, which may affect cell morphology. The method requires the use of morphological bacterial cells for further processing, the cell edge tracking and smooth and easy to extract. Due to the representation of the cell extract characteristic values are not uniform, while there is a complex relationship between the target output value and we can't use simple mathematical simulation methods. Therefore, a need for an efficient method for bacterial cells having common main component classification (Boyce and Havill, 2008). Figure 4 shows the image recognition system.

Currently used with a wide variety of image recognition technology in the field of image recognition, the most widely used artificial neural networks. Simply put, the artificial neural network is based on the imitation of biological structure and function of the brain, using the method of nonlinear network connection will be the information processing system research and composition. Having parallel structures and parallel processing capabilities, knowledge can be distributed memory, along with good fault tolerance, highly nonlinear and non-precision calculations, while the self-learning ability, self-organization and self-adaptive characteristics are also high. PCR method has higher specificity, sensitivity, quickness and convenience, which may provide a new approach to detect SA in food quickly.

Microscopic images of *Staphylococcus aureus* processed for feature extraction worth, the main advantage is the morphology of *S. aureus*, including perimeter, area, roundness, eccentricity, since the correlation between the morphology resistance, the final selection of the area, roundness, the eccentricity of the four characteristics of the cell as a characteristic feature recognition parameters *Staphylococcus aureus*, which is a self-employed capacity, fault tolerance and good BP neural network, the establishment of a *Staphylococcus aureus* recognition model. The model can accurately target cell will be classified and non-target cell recognition.

Rapid detection of *Staphylococcus aureus* in the application of computer vision technology, combined with the reduction of *S. aureus*-specific coloring

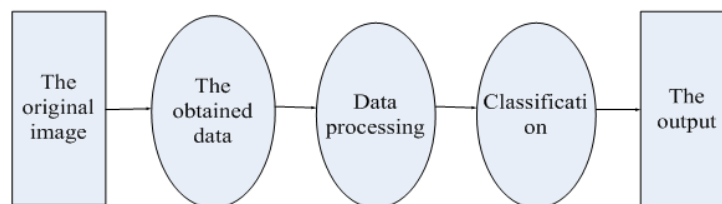


Fig. 4: The image recognition system

principle potassium and for the rapid detection of *Staphylococcus aureus* in food conducted a preliminary inquiry and have achieved some results. The laboratory research and development will be rapid microbial detection system used in total bacteria, coliform bacteria, Salmonella, Bacillus cereus detection also can be applied to the detection of *Staphylococcus aureus* bacteria. If this system is applied to on-site real-time monitoring of food and agricultural products can effectively control the situation of excessive microbial final product, reduce the incidence of food hygiene and safety incidents. And because of the diversity of the food, its texture is different and therefore need different types of food pre-treatment methods and classification to discuss and refine the test to verify and establish different types of food in the detection of *Staphylococcus aureus* pre-treatment method, a standardized pre-processing steps (Deurenberg and Stobberingh, 2008).

RESULTS AND DISCUSSION

***Staphylococcus aureus* detection method:** *Staphylococcus aureus* when extracting genomic DNA, the number of the obtained amount of template, amplification will affect the results. *Staphylococcus aureus* Gram-positive bacteria, cell wall thick, before DNA extraction, we must first be broken cell treatment. Large number of foreign data show that *S. aureus* when extracting DNA, mostly carried out using solvent broken wall hormone treatment. To determine the PCR method detection limit and different minimum initial amount of bacteria in food detection time, we introduce the direct plate count method in the study. Introduction plate count method enables PCR method can be carried out simultaneously in a qualitative semi-quantitative detection. PCR method established in this study is a high sensitivity, specificity and detection speed of approach is better than traditional detection methods. In theory, as long as the nucleic acid sequence is clearly limited, the use of PCR technology can detect any pathogens.

CONCLUSION

With the improvement of people's living standards, attention to food safety issues is also increasing, many scholars working on the rapid detection of pathogens in food. *Staphylococcus aureus* has been considered one of the most important bacteria causing food poisoning. In this study, the particularity of food PCR detection of *Staphylococcus aureus*, the establishment of a rapid detection of *Staphylococcus aureus* in Dairy PRC

method, eliminating the PCR reaction inhibition factor, shortening of *Staphylococcus aureus* in food testing time and improve detection methods sensitivity, provide technical support for the rapid diagnosis of *S. aureus* cause foodborne illness. Established by the PCR method to detect *Staphylococcus aureus* with fast, economical, sensitive and specific advantages, *S. aureus* in food could be detected by PCR in 24 h. The PCR method was faster than the culture method and provided a technical help to detect *S. aureus* in food rapidly.

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