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Full Length Research Paper

Obtaining DNA from *Staphylococcus aureus*: A study on DNA extraction methods for food matrices without bacterial isolation

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Polymerase chain reaction (PCR) is a tool widely used. However, for the effective use of PCR, DNA quality is necessary. Thereby, the objective of this study was to evaluate DNA extraction methods of *Staphylococcus aureus*, for direct application in food. Five methods were tested using reference and food strains. The methods show different characteristics with steps of thermal lysis, enzymatic lysis, detergents and organic solvent applications. The amplification was carried out using the primers COAG2 and COAG3 and the visualizations of the DNA extracted and amplification products were performed by gel electrophoresis. The method with SDS was not satisfactory using reference strain. The methods based on the use of cetyl trimethyl ammonium bromide (CTAB), sodium chloride-Tris-EDTA (STE) and enzymes showed positive results. Methods 2 (thermal lysis) and 3 (with CTAB) were used for DNA extraction from food samples, without bacterial isolation, and the PCR was subsequently performed. These methods were easier to implement and they show low costs. Thus, the methods 2 and 4 allowed the amplification of the DNA extracted from *S. aureus* from samples rich in protein and fat. Method 2 is practical and shows other advantages such as less manipulation of samples and reagents, nonuse of contaminant reagents and enzymes, less time for analysis, thus, lower costs.

Key words: Thermal lysis, DNA, quality, polymerase chain reaction.

INTRODUCTION

Staphylococcal food poisoning is considered one of the most common foodborne illnesses (Pelczar et al., 1996). The number of cases has reduced, but it is the third cause of food borne illness worldwide (Aydin et al., 2011).Thereby, the importance of *Staphylococcus aureus* in medicine has increased and supported by the development of bacterial resistance antimicrobials and the capacity to produce enterotoxins (Vasconcelos and Cunha, 2010).

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This pathogen is detected, in most cases, using microbiological culture and biochemical tests; however, these methods are not entirely satisfactory (Cremonesi et al., 2005) because they are associated with ambiguous results (Pinto et al., 2005). In the last decades, molecular methods have been developed and recognized for detection and characterization of microorganisms in foods. Molecular biology is a viable alternative to conventional methods for pathogen diagnostics in foods.

The PCR stands out due to its specificity and sensibility, speed, easy handling, and is a useful tool for routine application (Pinto et al., 2005). To obtain success in the reaction, DNA quality is necessary. The quality and yield of extracted DNA are critical for most applications of molecular biology (Minas et al., 2001). There are many DNA extraction kits, but they are expensive for small laboratories. Therefore, it is necessary to develop or adapt extraction methods.

Most extraction methods are based on sample and microorganism characteristics and promote cell rupture, cell components separation and DNA precipitation (Lima, 2008).

The microorganism isolation is common; however, bacterial isolation prior to extraction increases time and cost of analysis. Thus, the direct DNA extraction is proposed. The objective of this study was to evaluate methods of DNA extraction from *S. aureus* for direct application in food. The use of bacterial DNA extracted directly from food sample, without bacteria isolation, is the main point in this search.

MATERIALS AND METHODS

Samples

Firstly, strains of *S. aureus* ATCC 6538P and ATCC 25923 were used and *S. aureus* isolated from milk denominated SA1, SA2 and SA3, respectively.

The food samples were fresh sausage, Calabresa sausage, Toscana sausage, Blumenau sausage and fresh thin sausage marketed in the city of Campos Gerais, Paraná State, Brazil. The samples were not artificially contaminated.

Before the DNA extraction the strains were grown in brain heart infusion (BHI) broth for 24 h at 37°C. The samples were inoculated in buffered peptone water for 24 h at 37°C for a better growth of *S. aureus* and for the inhibition of competing microorganisms, 8.5 g of NaCl per liter of water was added. This material was used for the DNA extraction after the incubation time without the isolation of *Staphylococcus* sp.

DNA extraction

Five different methods were evaluated as below:

Method 1: using SDS (sodium dodecyl sulfate 1%), proteinase K, chloroform: isoamyl alcohol and ethanol (Moreira et al., 2010) modified;
Method 2: based on the boiling (thermal lysis) without the use of specific reagents, cited by Chapman et al. (2001);
Method 3: described by Chapaval et al. (2006), using CTAB (cetyltrimethylammonium bromide 2%), proteinase K, chloroform: isoamyl alcohol, ethanol and isopropanol;
Method 4: based on Millar et al. (2000), use of chloroform/isoamyl alcohol and DNA precipitation with ethanol;
Method 5: using lysozyme, proteinase K, STE (2.5% SDS, 10 mM Tris-HCl, 0.25 M EDTA), ammonium acetate, chloroform: isoamyl alcohol and isopropanol (Luz, 2008).

All methods were evaluated using the strains SA1, SA2 and SA3. The methods that showed potential for direct extraction were applied in samples food. The DNA extracted from samples food were used for amplification to evaluate the quality of the extracted material and the possible influence of the compounds food.

DNA amplification

The primers used were COAG2 (5'ACCACAAGGTACTGAATCAACG3') and COAG3 (5'TGCTTTCGATTGTTCGATGC3') described by Aarestrup et al. (1995) cited by Luz (2008). The size of the amplified product is approximately 800 pb, because of polymorphism in this species. The PCR for the gene coa detection was conducted in a solution containing 1x PCR buffer, 0.75 mM $MgCl_2$, 1 µM of each primer, 200 µM of dNTPs, 1.5 U of Taq polymerase and approximately 40 ng of DNA; final volume was completed to 25 µL with sterile water.

The amplification conditions were: initial denaturation at 95° C for 5 min followed by 40 cycles of 95°C for 30 s, 55°C for 2 min and 72°C for 4 min, and final extension at 72°C for 10 min (Luz, 2008). The amplification was carried out in a thermal cycler Axygen Maxigene®.

Visualization DNA and PCR products

The DNA quality was visualized by electrophoresis in 0.8% agarose gel. The amplicons were visualized by standard electrophoresis in 1.5% agarose gel, with molecular size marker (100 pb). The gels were immersed in ethidium bromide (0.5 µg/mL) for 15 min. For the band visualization, gel exposure was performed in a UV transilluminator and image capturing by software LPix Image.

RESULTS AND DISCUSSION

The Gram's method is an important parameter for DNA extraction, because it indicates the cell wall composition and, hence, its resistance (Nogueira et al., 2004).

Baratto and Megiolaro (2012) tested four DNA extraction methods for positive Gram bacteria and observed that the use of the SDS method allied to proteinase K obtained high quality and greater quantity of DNA when compared with methods using only SDS or boiling methods. In this study, method 1 (with SDS and proteinase K) was not effective for all strains (Figure 1a). This result may be associated with the characteristics and origin of the strains. Furthermore, previous studies showed SDS for DNA extraction from Gram-negative bacteria (Gonçalves, 2006). On the other hand, to confirm that DNA was not extracted is not correct because the quantity can be so little that it is not visualized on agarose gel.

The presence of defined bands is a reliable parameter to establish the method efficiency. However, Nogueira et al. (2004) determined that the absence of bands defined in agarose gels is not a predictive factor for the PCR success. The method based on microorganism boiling in water and centrifugation was indicated for several bacteria.

Method 2 is faster, simpler and cheaper than others under

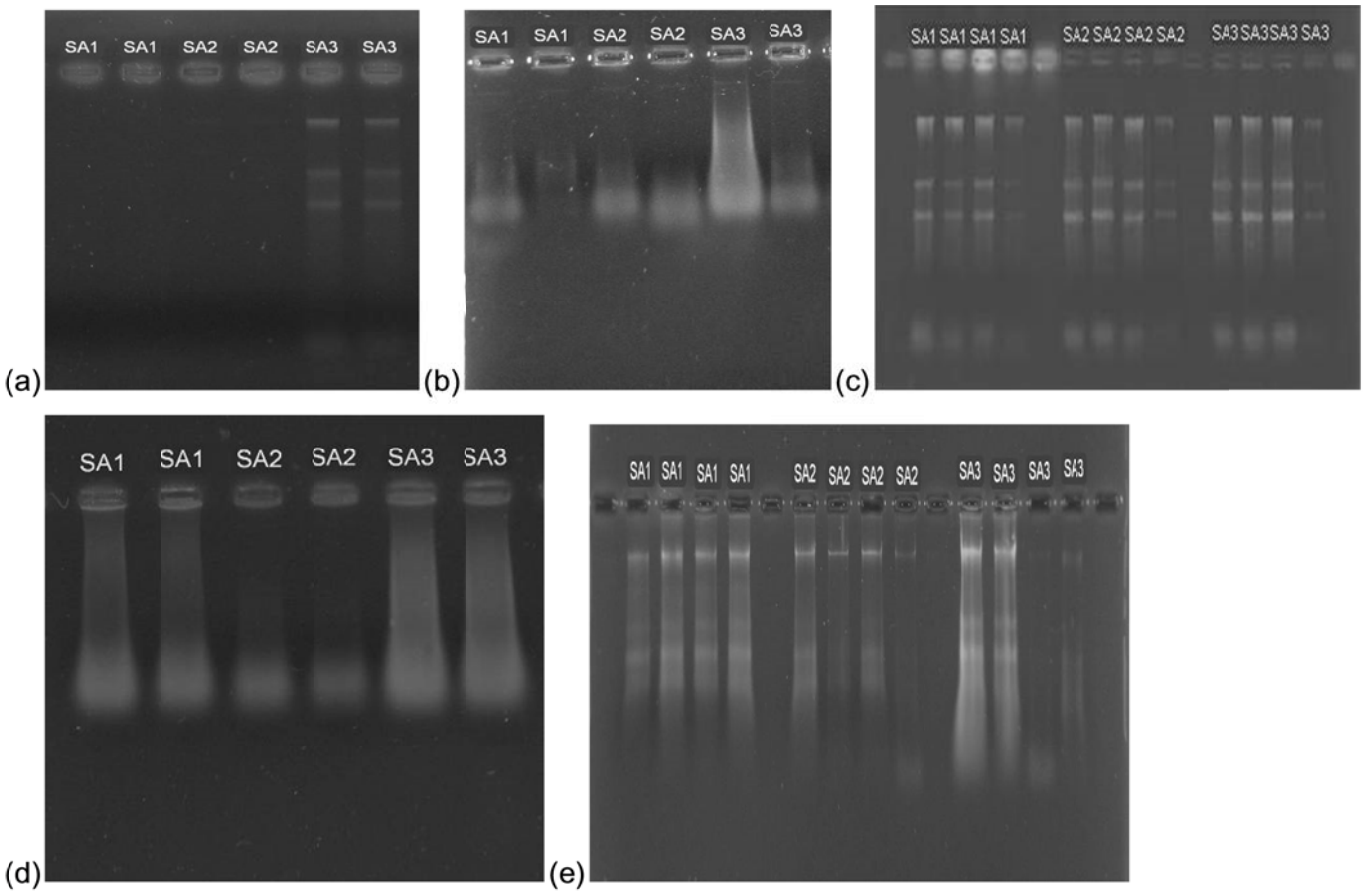

Figure 1. SA1: ATCC 6538P, SA2: ATCC 25923 and SA3: *S. aureus* isolated from milk. **(a)** Agarose gel with DNA extracted by Method 1. (b) Agarose gel with DNA extrated by Method 2. **(c)** Agarose gel with DNA extrated by Method 3. **(d)** Agarose gel with DNA extrated by Method 4; (e) Agarose gel with DNA extrated by Method 5.

discussion. But, the defined bands were not visualized (Figure 1b), because the DNA is only exposed and substances undesirables are present. Described by Chapaval et al. (2006) Method 3 was satisfactory (Figure 1c) for all strains, the CTAB is already widely used for DNA extraction from Gram positive bacteria Chapaval et al., 2006; Olivindo et al., 2009; Gonçalves et al., 2010; Minas et al., 2001).

The DNA obtained by Method 4 also showed no defined bands (Figure 1d); it was already used for detection of *S. aureus* directly from milk (Dias et al., 2011). Method 5 is ideal for *Staphylococcus* sp. (Figure 1e), but applies the use of various reagents and two enzymes, which increases the cost of the analysis.

Based on characteristics found, Methods 2 and 4 were selected for direct DNA extraction and subsequent PCR development due to low cost, although they showed no defined bands. If these methods do not present good results a next method would be tested. Thereby, after 24 h of incubation, the samples were subjected to DNA extraction and PCR.

The PCR results showed that the methods tested were able to extract DNA with adequate quality (Figure 2) and quantity; results were positive (coagulotypes with ~ 1000 bp). The result is noteworthy since the *S. aureus* DNA was extracted directly from food samples without cell isolation; these samples foods present high contents of protein, fat and seasonings, compounds that negatively affect in the PCR analysis.

Method 2 was considered the most suitable (Figure 2) because of the results and its advantages. This method presents advantages such as time of analysis, absence of the use of contaminant reagents and enzymes, less handling of samples and reagents and lower costs. Thus, Method 2 is indicated for direct extraction of food similar to those tested. However, the DNA extracted by this method is not suitable for storage, because rapid degradation occurs.

Conclusion

Methods employing CTAB, STE and enzymes showed positive results for DNA extraction from strains. Methods 2 and 4 were also tested in food products (sausages) and the DNA obtained were subjected to PCR, showing satisfactory results. In these cases, the DNA extraction of

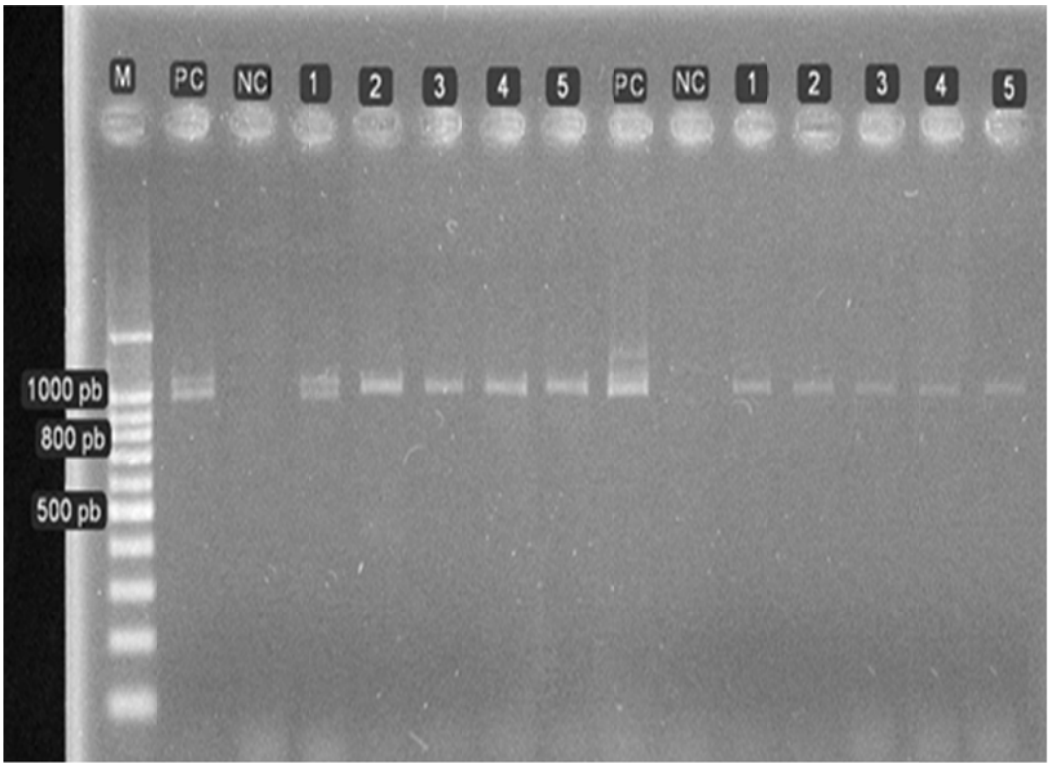

Figure 2. *coa* gene amplification from *S. aureus*. Line 2 to 8, DNA extraction by Method 2; Lines 9 to 15, DNA extraction by Method 4. M, molecular size marker; PC, Positive Control (ATCC 25923); NC, Negative Control (pure water); 1, fresh sausage; 2, Blumenau sausage; 3, Calabresa sausage; 4, fresh thin sausage; 5, Toscana sausage.

S. aureus directly from food in quantity and quality deserve highlight.

Diversified methods are available and the best choice should be linked to the quantity and quality of DNA extracted, analysis time, exposure to toxic reagents and costs. Thus, in this study, it can be concluded that Method 2 was the most suitable.

Conflict of interests

The authors have not declared any conflict of interest.

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