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PHAGE AMPLIFICATION ASSAY AS RAPID METHOD FOR *SALMONELLA* DETECTION

Regina Silva de Siqueira[1]*; Christine E.R. Dodd[2]; Catherine E.D. Rees[2]

[1]Embrapa Agroindústria de Alimentos, Rio de Janeiro, RJ, Brasil. [2]Division of Food Science, University of Nottingham, Sutton Bonington Campus, UK.

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ABSTRACT

The application of rapid methods is crucial for the HACCP program implantation in food industry. In this context, Phage Amplification Assay is a good candidate because is based on the interactions of phage and their host bacteria. This method using phage P22 was applied with to detect *Salmonella* cells in chicken breast. Samples of 25 g of chicken breast were diluted and the appropriate dilutions were used in phage amplification assay for *Salmonella* detection. After 3-4 h of incubation, it was observed a phage titre of approximately 10^4 pfu mL^{-1}, indicating that there were *Salmonella* cells which were naturally present in the meat. The presence of *Salmonella* cells were verified by using direct plating on XLD agar and by conventional enrichment procedure. The colonies suspected to be *Salmonella* were serologically tested and were identified as belonging to the serogroups B (*S. typhimurium* group) and D (*S. enteritidis* group). It can be concluded that this method provides a rapid and alternative application for *Salmonella* detection in food samples reducing both time and laboratory work to 3-4 hours.

Key words: *Salmonella*, rapid method, bacteriophage, P22.

INTRODUCTION

Salmonella is an important pathogen for the food industry and it has been a significant bacteriological agent of food-borne outbreaks. However, the whole protocol for their isolation and identification can take 3-6 days or more to yield a conclusive result. Thus, a number of procedures have been described which attempt to simplify the conventional method and reduce the elapsed time involved. The "Phage Amplification Assay" (PAA) was developed by Stewart *et al*. (4) and is based on the interactions of bacteriophage (phage) and their host bacteria, which can provide rapid and accurate detection of pathogens. This method exploits the lytic cycle of *Salmonella*-bacteriophage to indicate the presence of low levels of viable *Salmonella* cells in a sample within few hours. The aim of this study was to evaluate *Salmonella* phage P22 as an agent for detection of this pathogen by using PAA in chicken breast meat.

MATERIALS AND METHODS

Bacterial and bacteriophage strains

The bacteria strain used in this study was *Salmonella typhimurium* LT-2 (phage propagating strain) and the phage was P22. Phage stock was developed on its appropriate host strain by a plate lysis procedure (3).

Salmonella detection

25 g of chicken breast with skin were aseptically weighed and cut in small pieces, and ten-fold serial dilutions were prepared. The appropriate dilutions were used to detect *Salmonella* cells applying PAA and to streak out by direct plating on XLD. Additionally, a conventional enrichment procedure (CEP) in Rappaport-Vassiliadis broth, was carried out. Presumptive colonies on XLD were confirmed by serological tests.

*Corresponding author. Mailing address: Embrapa Agroindústria de Alimentos. Av. das Américas, 29.501, Guaratiba. 23.020-470, Rio de Janeiro, RJ, Brasil. Tel.: (+5521) 2410-7454. Fax: (+5521) 2410-1090. E-mail: siqueira@ctaa.embrapa.br

Phage Amplication Assay (PAA)

The method was carried out as described by Stewart *et al*. (4) as 10 mL of phage P22 carefully mixed with 100 mL of appropriate sample dilutions. The phage adsorption to bacterial cells was allowed at 37ºC/15 minutes, followed by the virucidal treatment for 5 minutes at room temperature. The virucidal activity was neutralized and the phage were amplified by adding helper bacteria from undiluted 18h culture. Finally, the mixture was transferred to molten top layer agar, poured onto TPA plate, and incubated at 37ºC/3-4h.

RESULTS

***Salmonella* detection by PAA**

The PAA was used to detect *Salmonella* cells in chicken breast meat. The results of PAA are shown in Table 1. It can be observed that the number of pfu mL^{-1} recorded was approximately 10^4 pfu mL^{-1} indicating probably the actual number of bacteria cells since the number of the plaques obtained is correlated to the number of cells in the sample.

Direct plating on XLD and conventional enrichment procedure

Some small, round and red colonies were observed on XLD by direct plating whilst red colonies without black centers were observed from CEP. These characteristics are typical of *Salmonella* colonies on this agar as described by Andrews *et al*. (1) The isolates suspected to be *Salmonella* were confirmed serologically by *Salmonella* agglutinating antisera. Most of the isolates were in the *S. enteritidis* group (D). From CEP, the three isolates belonged to *S. typhimurium* group (B) and two isolates belonged to *S. enteritidis* group (D).

Table 1. Detection of *Salmonella* cells in chicken breast applying PAA.

Control Chicken Sample	Number of pfu ml^{-1} obtained
Sample I	3.15×10^4
Sample II	5.10×10^4
Sample III	1.49×10^5

DISCUSSION

Detection of *Salmonella* cells by PAA was investigated. The effectiveness of this method is determined by comparing the number of plaques produced on a lawn of helper bacteria with the number of colonies produced from equivalent sample. From the results of PAA was observed the presence of 10^4 pfu mL^{-1} and from counting only (data not shown) indicated approximately 10^4 cfu g^{-1} were present in the sample. Thus, it was shown to have a good correlation between the number of plaques observed and the number of cells contained in the sample. From direct plating on XLD, the presence of *S. enteritidis* serogroup (group D) was found. These findings were supported by Baxa *et al.* and who reported that phage P22 is able to infect *Salmonella* serogroups A, B and D.

CONCLUSION

Phage P22 was able to infect bacterial cells of *S. typhimurium* and *S. enteritidis*. The Phage P22 Amplification Assay can be successful applied and is a good candidate to be used as alternative rapid method.

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RESUMO

Amplificação de bacteriófagos como um método rápido de detecção de *Salmonella*.

A aplicação de métodos rápidos é crucial para a implantação de programas de HACCP em indústrias de alimentos. Neste contexto, o método de amplificação de bacteriófagos é um instrumento de diagnóstico importante porque está baseado na interação dos bacteriófagos com suas células hospedeiras. Este método, usando o bacteriófago P22, foi aplicado para detectar *Salmonella* em peito de frango. Amostras de 25 g de peito de frango foram diluídas e as diluições apropriadas foram usadas no método de amplificação de bacteriófagos na detecção de *Salmonella*. Após 3-4 horas de incubação, foi observado uma titulação de partículas virais de, aproximadamente, 10^4 ufp mL^{-1} (unidades formadoras de placas virais), indicando a presença de células de *Salmonella* na carne de frango. A comprovação da presença de *Salmonella* neste produto foi verificada usando-se plaqueamento direto em ágar XLD e procedimento de enriquecimento convencional. As colônias suspeitas de *Salmonella* foram sorologicamente testadas e identificadas como pertencendo aos sorogrupos B (grupo de *S. typhimurium*) e D (grupo de *S. enteritidis*). Portanto, concluiu-se que este método pode ser aplicado, na detecção de *Salmonella* em alimentos, porque fornece rápido e conclusivo resultado, reduzindo o tempo de análise e o trabalho laboratorial para 3-4 horas.

Palavras-chave: *Salmonella*, método rápido, bacteriófago, P22.

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