ASSESSING THE EFFECT OF HEAT TREATMENT ON ANTIMICROBIAL RESISTANCE GENES, BACTERIAL TRANSFORMATION AND BACTERIOPHAGE TRANSDUCTION.

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INTRODUCTION

The widespread use of antibiotics in the livestock and agricultural industry has contributed to the transmission of antimicrobial resistance (AMR) and presence of antimicrobial resistant bacteria (AMB) in retail meat. The role of retail meat in the transmission of AMR through AMB and antimicrobial resistance genes (ARGs) is not fully known. A recent study has demonstrated that the horizontal gene transfer (HGT) of ARGs can occur between different bacterial species within a food matrix. Furthermore, ARG-carrying bacteriophage have been isolated from various food products and have been shown to transduce resistance to antimicrobials. Cooking regimes have been proven to inactivate and destroy bacteria, however their effects on bacteriophage, bacterial DNA and HGT within food products is largely unknown. Therefore, this study aims to investigate the impact of heat, modelled after various core cooking temperatures of meat, on ARGs, bacterial transformation, and phage transduction, in order to understand how cooking may affect ARGs and HGT. This study also investigated the effect of heat treatment at 121°C on the integrity of ARGs and DNA fragmentation.

METHODOLOGY

Chemically competent Escherichia coli DH5α were transformed with pUC19 according to Invitrogen's recommended transformation procedure. pUC19 was isolated and purified from successfully transformed colonies. E. coli K12 ER2738 were infected with M13 phage. Successfully transduced colonies were used to amplify and purify a solution of M13 phage. Solutions of pUC19 and M13 phage were heat treated at 50°C, 65°C, 75°C, 80°C and 95°C for two minutes and at 121°C for 15 minutes. To assess the impact of heat on antimicrobial resistance genes, cultures of bacterial isolates E. coli K12, E. coli NCTC 12241 and E. coli DH5α with pUC19 were heat treated at 121°C for 15 minutes. PCR was conducted on heat treated E. coli K12 and NCTC 12241 for the presence of the whole ampC gene and its promoter.

RESULTS

Heat treatment at 50°C, 65°C, 75°C, 80°C and 95°C for two minutes resulted in less than a 1-log reduction in the transformation efficiency of pUC19 and M13 phage transduction. The heat treatment of pUC19 at 50°C, 65°C, 75°C, 80°C and 95°C for two minutes did not result in a significant decrease ($p>0.05$) in transformation efficiency. M13 phage heat treated at

50°C, 65°C, 75°C, 80°C and 95°C for two minutes and 121°C for 15 minutes did result in a significant decrease in transduction ($p<0.01$). The heat treatment of bacterial isolates, pUC19 and M13 phage at 121°C for 15 minutes resulted in DNA fragmentation of the ampC gene and loss of pUC19 transformation, while M13 phage retained viability in low numbers (1.1x105 pfu/ml, 1.3x105pfu/ml). In conclusion, this study showed that pUC19 and M13 phage were able to retain transformation and transduction abilities when heated at 50°C, 65°C, 75°C, 80°C and 95°C for two minutes. Furthermore, heat treatment at 121°C for 15 minutes did not destroy bacterial DNA and antimicrobial resistance genes but resulted in DNA fragmentation.

DISCUSSION

The results of this study indicate that pUC19 and M13 phage can withstand temperatures up to 95°C for 2 minutes. PCR carried out on heated bacterial isolates, E. coli K12 and NCTC 12241, for the ampC gene and its promoter showed the promoter region of the gene to be present whereas the full gene was not; indicating that the gene was fragmented but not destroyed. A previous study has shown that fragmented and damaged DNA can be uptaken by bacteria and lead to their transformation. In the case of the ampC gene, where mutations in the promoter region lead to increased production of AmpC cephalosporinase, the uptake of the fragmented promoter by viable bacteria could potentially lead to the incorporation of the fragment into bacterial DNA, increasing AMR. Further research is needed to understand if fragmented ARGs can lead to the transformation of viable bacteria and the transmission of AMR. Overall, findings from this study suggest that internal cooking temperatures do not result in the inactivation of plasmid mediated bacterial transformation and phage transduction, or in the destruction of ARGs, suggesting continued ARG transmission could occur through HGT within a food matrix post cooking.