Evaluation of false-positive PCR signals due to free DNA in food samples

Petra Wolffs and Peter Rådström

Applied Microbiology, Center for Chemistry and Chemical Engineering, Lund Institute of Technology, Lund University, P.O. Box 124, SE-221 00 Lund, Sweden, Phone: +46 46 222 06 49, Fax: +46 46 222 42 03, E-mail: petra.wolffs@tmb.lth.se

Abstract: To assess the risk of false-positive signals in PCR, caused by DNA originating from dead cells, the natural degradation of DNA in food samples was studied. A sample preparation method was developed in connection with real-time PCR to quantify the degradation of DNA in carcass-rinse from chicken and pork homogenate. Our results indicate that the risk of obtaining false-positive PCR signals is significant in pork homogenate, since DNA degradation is slow, but less pronounced in carcass-rinse from poultry.

Keywords: Pork homogenate, Chicken Carcass-rinse, false-positive PCR signals

Introduction: PCR is frequently used as a diagnostic tool for the detection of pathogens in food samples. Where PCR offers advantages like decrease in detection time and possibilities for automation, some challenges remain. Where traditional microbiological methods detect the living organisms, PCR detects DNA. The presence of DNA is however not an indication of the presence of living cells and can therefore lead to false-positives in detection. Recent research has mainly focussed on the detection of bacterial mRNA as an indicator of living cells. However recent research has already indicated that the natural degradation of DNA in food can be rapid (Nogva et al, 2000). The aim of this work is to study the natural degradation of DNA in pork homogenate and carcass rinse of poultry by using quantitative PCR.

Materials and Methods: DNA was purified from overnight cultures of Yersinia enterocolitica Y 79 (Norwegian Meat Research Centre, Oslo, Norway) using the EasyDNA kit (Invitrogen, Groningen, The Netherlands). Pork homogenate samples were made by adding 25 g minced pork meat into 225 ml physiological saline and homogenising for 10 min in a stomacher. For carcass rinse from poultry, 25 g neck skin from chicken was added to 225 ml physiological saline and homogenised for 10 min. After stomaching the skin was removed from the sample. Real-time PCR was performed using the yadA primer pair from a multiplex PCR assay developed.
by Lantz et al. 1998. The PCR mixture was composed of 0.4 \mu M of each primer, 0.2 mM of each dNTP, 4 mM total Mg\(^{2+}\) concentration and 2.5 U DNA polymerase with its accompanying buffer. Finally 1 \mu l of 10,000 times diluted SYBR Green I (Roche Molecular Biochemicals, Basel, Switzerland) was added to the reaction volume, which resulted in 16 \mu l total volume. To this 4 \mu l sample was added. The PCR consisted of 1 min initial denaturation at 94 °C, followed by 40 cycles of 0.1 s of denaturation, 5 s annealing at 58 °C and 25 s of elongation at 72 °C, followed by a single fluorescent measurement. After the 40 cycles a melting curve analysis was run from 65°C to 95°C. After amplification the Cp value (crossing point value) was determined, using the second derivative method of the LightCycler software version 3.0. The Cp value was plotted against the log concentration, to create a standard curve for determination of the DNA concentrations. The identity of the PCR products was confirmed using melting curve analysis and traditional 1.3 % agarose gel electrophoresis. All results are obtained from at least duplicate measurements.

Results and Discussion: In order to investigate the PCR inhibition of the carcass-rinse from chicken and the pork homogenate, both were included in the PCR mixture in a series of tenfold dilutions. Taq polymerase and Tth polymerase were tested for their susceptibility to the inhibitors in both food samples. The results show that both enzymes are inhibited in almost all cases by the undiluted food samples (data not shown) Furthermore it becomes clear that Tth polymerase is less inhibited by both food samples and that a tenfold dilution of the food-sample combined with the use of Tth will allow direct detection in the samples.

The natural degradation of DNA in food samples was studied by following the degradation of the DNA with real-time PCR. An increase in Cp value implies a decrease in DNA concentration. The results show clearly that a degradation of DNA in the food samples was taking place (figure 1). When the DNA degradation was studied in both food samples it becomes also obvious that DNA is much more stable in pork homogenate than in carcass-rinse from chicken. Reasons for that can be found in the different type of sampling. The pork homogenate still contains the original meat sample, whereas in the carcass-rinse the food sample is removed after stomaching. That implies a high presence of protein in the food sample and proteins have been shown to stabilise DNA and facilitate amplification (Abu al-Soud, 2000).
Figure 1: The degradation of DNA (0.1 mg/ml) in pork homogenate and chicken carcass-rinse. + : signal with gel electrophoresis and melting curve analysis, - : no signal with gel electrophoresis and melting curve analysis

It can be concluded that we have developed a way for direct analysis of the food samples by tenfold dilution and the use of the alternative DNA polymerase Tth. Furthermore it can be concluded that the DNA degradation in carcass-rinse from chicken is rapid, and slow in pork homogenate. This means that the risk for obtaining false-positive results in the PCR detection on living bacteria is present, mainly in pork homogenate. Further studies will need to be done to study the effect of the sampling on the stability of the free DNA in the food samples, since there are indications that rinse or wash techniques might lower the risk of obtaining false-positive signals with PCR.

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