QUANTIFICATION OF TOTAL VIABLE COUNTS (TVC'S) ON FRESH MEAT CARCASSES BY A NOVEL REAL-TIME POLYMERASE CHAIN REACTION

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Introduction

The safety of food must be assured by a preventative approach based on the application of a food safety management system such as Hazard Analysis Critical Control Points (HACCP) and setting of microbiological criteria. Testing of samples against these criteria can be used to provide assurance that process controls, performance criteria or food safety objectives have been achieved (Stewart *et al.*, 2002). The total viable count can be performed by a cultural method (ISO 4833 which takes 3 days) or by an alternative rapid method if shown to be equivalent (ISO 16140:2003). The aim of this project is to develop and apply highly specific and user friendly nucleic acid based (DNA and RNA) rapid methods based on Real-Time PCR for the determination of total viable counts on fresh meat carcasses. Subsequently a procedure for demonstration of equivalence based on that outlined in EN/ISO:16140 will be followed. As it is essential that only viable organisms be detected, Reverse Transcriptase PCR which detects specific RNA sequences (only expressed by viable cells) will be employed. It will be applied in a Real-Time (Lightcycler) automated PCR format, which uses fluorescence to detect the presence and quantity of a particular gene in real time thus making it make more rapid and user friendly than conventional PCR (Yaron and Matthews, 2002).

Materials and Methods

In order to establish the microflora of a beef carcass, 90 slaughtered steers were sampled over a 5 week period in an Irish abattoir. Each carcass was sampled using the sponge swab technique of four sites (neck, flank, brisket and rump) before chilling as outlined in the EU 471 directive. The samples were stored at 4°C until further processing on the same day. Samples were plated onto Plate Count Agar (PCA) to establish the total viable count (TVC) and on a range of different agar bases in order to identify particular species that were typical or dominant. Following sampling, 40 bacterial colonies were selected at random for identification by 16S rDNA sequencing.

Using the information obtained from the sequencing, pure bacterial cultures typical of those occurring on beef carcasses were selected. DNA was extracted from pure cultures grown overnight at the appropriate temperature using the DNeasy® Tissue Kit (Qiagen, Hilden, Germany), according to the manufacturers instructions. For the PCR, a master mix of total volume 45µl was prepared, to which 5µl of DNA was added. The master mix contained novel primers for a specific gene target common to all bacteria. The PCR was carried out on a DNA Engine Dyad™. At present, the assay is being transferred to a real-time PCR platform on the Roche LightCycler®.

Results and Discussion

The predominant microbial flora at the pre-chilling stage of the slaughter process have been shown to be Lactic Acid Bacteria and *Enterobacteriaceae* spp. In addition, a broad range of Gram-positive and Gram-negative bacteria were detected, including *Acinetobacter* spp., Staphylococcae and *Pseudomonas* spp. In the PCR reaction, designed primers were tested against 40 bacterial isolates, and the expected region was amplified in all species (Figure 1). The selected gene is highly expressed in all bacteria and therefore will be very amenable to an RNA based reverse transcriptase PCR assay. Once this target has been fully characterised, it will be assessed for the ability to be used as a working assay for total viable counts using the above outlined technology.

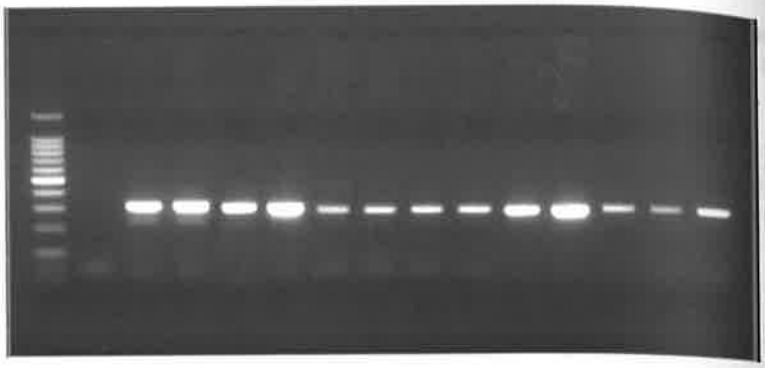

Figure 1: 2% agarose gel showing the amplicon obtained in the PCR reaction. Lane: 1. 100 base pair ladder (Promega Corporation, WI, USA); 2. Negative control; 3. *Acinetobacter calcoaceticus*; 4. *Aeromonas hydrophila*; 5. *Aeromonas salmonicida*; 6. *Enterobacter aerogenes*; 7. *Enterococcus faecium*; 8. *Enterococcus gallinarum*; 9. *Lactobacillus plantarum*; 10. *Leuconostoc mesenteroides*; 11. *Pseudomonas flourescens*; 12. *Pseudomonas putida*; 13. *Staphylococcus epidermis*; 14. *Staphylococcus haemolyticus*; 15. *Staphylococcus saprophyticus*.

Conclusions

Based on these results it may be concluded that this method seems to be adaptable to a real-time PCR assay to quantify total bacteria on fresh meat carcasses. An alternative rapid method, which gives results within a few hours, would be of immense benefit to the meat industry, allowing real time assessment of product quality and reduced holding time of the product.

References

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