International Research Grant report

The effect of PCR inhibitors in complex food samples

I was fortunate to be awarded an International Research Grant to visit the laboratory of Dr Cath Rees and Dr Christine Dodd at the Food Science department of the University of Nottingham from May to August 2004. My research centres on food safety, food-borne pathogens, diagnostics and preservation. The focus is on the development and improvement of rapid DNA methods for the detection and quantification of pathogenic bacteria in food and water.

Listeria is an important human food borne pathogen. Conventional methods to detect it in foodstuffs are generally cumbersome and time-consuming. The polymerase chain reaction (PCR) is a highly sensitive, specific and rapid method for detection of bacteria in pure cultures, but the low concentration of pathogens and the presence of PCR inhibitors in complex food samples will reduce the amplification or even block DNA synthesis. The approach that we used was to target the hlyA of *Listeria monocytogenes* to obtain 730 bp PCR product. Different pre-PCR treatments were developed to concentrate DNA or target cells, and to counteract the effect of PCR inhibitors. Different DNA polymerases, including *Taq* and *Tth* were evaluated, with different PCR buffers and different PCR facilitators.

It was found that different enrichment broths inhibited the PCR reaction when *Taq* DNA polymerase was used, but to our surprise, when we used *Tth* DNA polymerase there was no or little inhibition of the PCR reaction.

Staphylococcus aureus is a ubiquitous Gram-positive coccus, of which many strains contribute to the body's natural flora. Although not normally associated with acute disease, *S. aureus* does possess numerous potent virulence factors and, given the opportunity, can be the causative agent of serious illnesses such as osteomyelitis and endocarditis.

S. aureus is able to express a variety of extracellular toxin proteins, including toxic shock syndrome toxin 1 (TSST-1) and enterotoxins. Staphylococcal enterotoxins (SEs) encoded by the *ent* genes are the causative agents of staphylococcal food poisoning.

The aim of this part of the study was to optimize the PCR conditions for the universal forward and reverse primers to enable the reaction to be used as a universal enterotoxin detection test. The universal primers targeted conserved domains in each of the genes; therefore, the size of PCR product would be expected to be the same but with differing nucleotide sequences. To determine the number and nature of the genes present, DGGE profiles of the amplicons were produced. In this way the PCR reaction coupled with DGGE analysis had the potential for differentiating enterotoxin-positive and -negative strains of *S. aureus* and also between potential new genes in strains where no genes were previously identified.

My research visit was a fantastic experience, as it allowed me to test a few ideas in the laboratory, build collaboration and make friends. I would like to thank the SGM for awarding the grant, and Cath and Christine, as well as all the members of their labs, for making it easy for me to settle in. I would also like to acknowledge the helpful support of Dr Sara Movahedi and David Fowler.

Pieter Gouws
University of the Western Cape, South Africa (**e** pgouws@uwc.ac.za)

▲ Coloured transmission electron micrograph of *Listeria monocytogenes*. *Moredun Animal Health Ltd / Science Photo Library*

◀ The author in the lab in Nottingham. *P. Gouws*