NEW MICROBIOLOGICA, **29**, 261-267, 2006

Growth of *Salmonella enterica* in model mixed cultures during a two-step enrichment

Klára Krascsenicsová, Eva Kaclíková, Tomáš Kuchta

Department of Microbiology and Molecular Biology, Food Research Institute, Bratislava, Slovakia

SUMMARY

Growth of *Salmonella enterica* was studied in model mixed cultures with *Citrobacter freundii* or *Escherichia coli* in buffered peptone water (BPW) and in Rappaport-Vassiliadis medium with soya (RVS) with modified concentrations of $MgCl_2$ and malachite green, and at modified incubation temperatures. Selected *S. enterica* strains were inoculated in BPW (10^0 cfu/ml) together with selected strains of *Citrobacter freundii* (up to 10^8 cfu/ml) or selected strains of *Escherichia coli* (up to 10^8 cfu/ml), incubated overnight and then subcultured (1: 100) in RVS variants. Growth of individual bacterial species was followed by the quantitative real-time polymerase chain reaction (PCR). Optimal culture conditions during the second selective step were: $MgCl_2.6\ H_2O$ concentration of 29 g/l, malachite green concentration of 36 mg/l, and the incubation temperature of 41.5 °C. *Citr. freundii* was found to be a potent competitor and *E. coli* was a weaker competitor. At optimal culture conditions, competition was reduced and the density of *S. enterica* cultures reached the level of 10^4 cfu/ml after not later than 2 h of selective enrichment. The results obtained provide a basis for the development of a short two-step enrichment to be used in rapid real-time PCR-based methods for the detection of *S. enterica* in food and other matrices.

KEY WORDS: Salmonella, Rappaport-vassiliadis medium with soya (RVS), Real-time polymerase chain reaction (PCR), *Citrobacter*, *Escherichia coli*

Received June 15, 2006 *Accepted* August 16, 2006

INTRODUCTION

Salmonella enterica is a food-borne pathogen which represents a major public health problem in almost all industrialized countries (Tietjen *et al.*, 1995). For routine detection of *S. enterica* in food and other matrices, a conventional method according to EN ISO 6579 (2002) is used. This standard method is sensitive and reliable but requires up to 7 days. Recently, a number of rapid methods for the detection of *S. enterica* in food have been developed including immunoassays and polymerase chain reaction (PCR) techniques. The best parameters were reported for methods based upon real-time PCR which detected the pathogen at the level of 10^0 cfu / 25 g in a closed-tube format (Uyttendaele *et al.*, 2003; Malorny *et al.*, 2004; Patel *et al.*, 2006).

Most currently available real-time PCR-based methods for the detection of *S. enterica* in food include an overnight pre-enrichment in buffered peptone water (BPW). This simple step seems to provide a sufficient amount of *S. enterica* for downstream detection (Malorny *et al.*, 2004). Nevertheless, the single-step enrichment may produce false positive results caused by the presence of dead bacterial cells in the sample (Drahovská *et al.*, 2001) or false negative results caused by food-borne PCR inhibitors (Salomonsson *et al.*, 2005). These problems may be solved if a two-step enrichment is used. In this case, dead bacterial cells as well as food-borne PCR inhibitors would be diluted and would not affect the results

Corresponding author
Tomáš Kuchta
Food Research Institute
Priemyselná 4, PO Box 25,
SK-82475 Bratislava 26, Slovakia
EE-mail: kuchta@vup.sk