SALMONELLA-PCR: SCREEN FOR MICROBIOLOGICAL SAFETY

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SUMMARY

We present the development and application of (IMS-)PCR methods for routine detection of *Salmonella* in specific food matrices (figure 1). Enrichment techniques, Polymerase Chain Reaction (PCR) and optionally Immunomagnetic Separation (IMS) were combined into fast screening methods adapted to the particular food matrix and the specific requirements of the PCR. The performance of these methods was evaluated in implementation studies with artificially contaminated and fresh samples from the meat production line, and in field trials at plant laboratories. Correlation between the results of the PCR methods and the standard detection method was more than 98%. Inclusion of IMS into the PCR method improved the intensity of the PCR signals and, for samples with high levels of competing flora or PCR disturbing components, the sensitivity of the detection method. The total detection time might be further reduced with even shorter enrichment and/or IMS prior to PCR.

Probe and primer development

Probes were selected from a library of randomly cloned DNA fragments (*Salmonella* subclass I) by differential hybridization with chromosomal DNAs from *Salmonella* and non-*Salmonellae*. For one of these probes the complete nucleotide sequence was determined and the best conserved region was selected. After alignment of the homologous sequences of this region in all other *Salmonella* subclasses several sets of *Salmonella*-specific PCR primers were designed. Confirmation of the PCR results can be performed either by hybridization or by a second PCR with internal primers. The PCR primers were tested on *Salmonella* strains from all subclasses and non-*Salmonellae*. All *Salmonellae* were detected, and no false positives were found.

An internal control, consisting of a genetically modified region of the *Salmonella*-specific probe, was added to the samples before PCR analysis. Amplification of this control DNA with the *Salmonella*-specific primers results in a PCR product which is larger than the *Salmonella*-specific fragment (figure 2). False negative PCR results as a consequence of technical failures in the PCR assay can be deduced from the absence of PCR product. Presence of low levels of *Salmonella* in the sample results in two amplification products, i.e. of the *Salmonella* DNA and the internal control. In high contaminated samples only the *Salmonella* fragment will be found after PCR, whereas only the amplified internal control will be detected in uncontaminated samples.

Development of (IMS-)PCR methods

For implementation of our specific PCRs we focused on meat and meat products as well as on swab samples from pig carcasses and slaughterhouse equipment. Three (IMS-)PCR methods for routine detection of *Salmonella* in the meat production line were developed (figure 3). Simple enrichment and sample pretreatment steps have been combined with a *Salmonella*-specific PCR and optionally IMS. For the enrichment protocols we used internationally recognized culture media. The sample pretreatment before PCR consisted of simple dilution or centrifugation steps to remove PCR inhibiting components from meat and media. In these studies the results were visualized by agarose gel electrophoresis and ethidium bromide staining. Modification of the PCR will allow replacement by other visualization techniques in the near future, e.g. colorimetry.

The first PCR method combines standard enrichment with PCR. Results are available after 44 hours. Since both standard detection method and PCR method can be carried out from the same sample of 25 gram this PCR method offers an ideal starting point for introduction of PCR techniques in the field. Modification of the standard enrichment into a 20-hours culturing step resulted in a 24-hours PCR method. With IMS included (IMS-PCR method) results are available after 25 hours and intenser PCR signals are found for artificially contaminated meat products (figure 4). Detection levels were estimated at < 10 cells/swab (pigcarcasses) and 1 cell/25 grams (meat products, even after storage at -20°C for 15 days, table 1).

Implementation

In an implementation study swabsamples from fresh pigcarcasses and slaughterhouse equipment were screened with the 44-hours PCR method, the 24-hours PCR method and the standard *Salmonella* detection method. Correlation between the results of the different methods was ≥ 98%.

Experimental field trials for the three (IMS-)PCR methods were conducted in a slaughterhouse and a meat product factory with 216 samples. All experiments were carried out at the plant laboratory and the samples were investigated with both standard detection methods and (IMS-)PCR methods. Special attention was paid to the simplicity of the methods for routine laboratories.
Salmonella was detected in 50 samples by (IMS-)PCR and all results could be confirmed by isolation of the microorganism. 3 samples were found negative by the standard method but positive by (IMS-)PCR. From two negatives saccharose-fermentative *Salmonella* could be isolated after reinvestigation of the samples with standard techniques. In the third sample *Salmonella* appeared to be overgrown by competing flora on cultureplates and could be isolated after reinvestigation of the sample.
The performance of the developed methods will be statistically evaluated by comparison of the results with the reference method for the detection of *Salmonella* in food (ISO 6579/DIS 3565) for large numbers of fresh samples.

Conclusions:

- reduction of the time for *Salmonella* detection to 24-25 hours with a PCR based screening method
- improved PCR signal and sensitivity when IMS is included into the PCR method
- ≥ 98% correlation between the standard detection method for *Salmonella* and the (IMS-)PCR methods

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