Direct PCR - A rapid method for multiplexed detection of different serotypes of Salmonella in enriched pork meat samples - DTU Orbit (09/11/2018)

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Salmonellosis, an infectious disease caused by Salmonella spp., is one of the most common foodborne diseases. Isolation and identification of Salmonella by conventional bacterial culture method is time consuming. In response to the demand for rapid on line or at site detection of pathogens, in this study, we developed a multiplex Direct PCR method for rapid detection of different Salmonella serotypes directly from pork meat samples without any DNA purification steps. An inhibitor-resistant Phusion Pfu DNA polymerase was used to overcome PCR inhibition. Four pairs of primers including a pair of newly designed primers targeting Salmonella spp. at subtype level were incorporated in the multiplex Direct PCR. To maximize the efficiency of the Direct PCR, the ratio between sample and dilution buffer was optimized. The sensitivity and specificity of the multiplex Direct PCR were tested using naturally contaminated pork meat samples for detecting and subtyping of Salmonella spp. Conventional bacterial culture methods were used as reference to evaluate the performance of the multiplex Direct PCR. Relative accuracy, sensitivity and specificity of 98.8%; 97.6% and 100%, respectively, were achieved by the method. Application of the multiplex Direct PCR to detect Salmonella in pork meat at slaughter reduces the time of detection from 5 to 6 days by conventional bacterial culture and serotyping methods to 14 h (including 12 h enrichment time). Furthermore, the method poses a possibility of miniaturization and integration into a point-of-need Lab-on-a-chip system for rapid online pathogen detection.

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