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Review

FISHing for bacteria in food – A promising tool for the reliable detection of pathogenic bacteria?

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ABSTRACT

Foodborne pathogens cause millions of infections every year and are responsible for considerable economic losses worldwide. The current gold standard for the detection of bacterial pathogens in food is still the conventional cultivation following standardized and generally accepted protocols. However, these methods are time-consuming and do not provide fast information about food contaminations and thus are limited in their ability to protect consumers in time from potential microbial hazards. Fluorescence *in situ* hybridization (FISH) represents a rapid and highly specific technique for whole-cell detection. This review aims to summarize the current data on FISH-testing for the detection of pathogenic bacteria in different food matrices and to evaluate its suitability for the implementation in routine testing. In this context, the use of FISH in different matrices and their pretreatment will be presented, the sensitivity and specificity of FISH tests will be considered and the need for automation shall be discussed as well as the use of technological improvements to overcome current hurdles for a broad application in monitoring food safety. In addition, the overall economical feasibility will be assessed in a rough calculation of costs, and strengths and weaknesses of FISH are considered in comparison with traditional and well-established detection methods.

1. Introduction

Zoonotic infections due to the consumption of contaminated food are still a global public health concern. Although significant efforts have been undertaken to limit the extent of foodborne bacterial infections by the implementation of higher hygiene standards and intensive testing, the number of infections remains high. In the European Union (EU), approximately 350,000 cases of foodborne bacterial infections were reported in the year 2011 (EFSA and ECDC, 2013). The estimated number of unreported cases per annum is considerably higher. *Campylobacter* spp. and *Salmonella* (*S.*) *enterica* as the most prominent pathogens might be responsible for more than 15 million cases in the EU (Havelaar et al., 2013). Likewise, acute and severe diarrhoea, with bacteria being major causative agents as well as viruses and parasites, is responsible for the death of about 1.2 million children under the age of 5 per year worldwide and a total of 1.6 billion cases of disease (Fischer Walker et al., 2012; Walker and Black, 2010). Therefore, food safety and the fast detection of frequent bacterial pathogens, for instance *Campylobacter*, *Salmonella*, *Listeria*, *Escherichia coli*, *Shigella*, *Vibrio* and *Yersinia*, are still important issues throughout the world and will also in the future retain its importance in the food industry and microbiological quality control.

The threat posed by foodborne pathogens can be monitored by a range of different detection methods. Some of them enable only the qualitative confirmation for the presence or absence of a pathogen, while others allow also for the quantification of the bacterial load (López-Campos et al., 2012). The latter feature might not be of significance for pathogens with a zero-tolerance-standard, but is especially important for pathogens which are acceptable in food products if the concentration is below a certain limit. Conventional plating and cultivation of pathogenic bacteria is still the method of choice and the gold standard to assess the degree and extent of contaminations in a variety of food products (Ge and Meng, 2009). In addition, reliable quantitative methods like the determination of the most probable number (MPN) are available as well as several modifications of standard protocols and growth media (Jasson et al., 2010). The high food safety requirements are met by the great sensitivity of these cultural methods. However, these techniques with their well-established, standardized and broadly accepted protocols are time-consuming, tedious, labour-intensive and often expensive (Velusamy et al., 2010). Depending on the

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pathogen, it can take several days up to weeks to verify preliminary positive results. Furthermore, it may be difficult to recover all the sublethally injured microbes out of the food matrix in case of pathogens which have encountered rather unfavourable conditions outside their natural habitat and might enter a state with low metabolic activity in which cell division is stalled (Oliver, 2005, 2010). The often employed non-selective preenrichment might be helpful under these circumstances, but this procedure also prolongs the overall analysis time. The increase in global trade and the associated need for a fast transport of food products over large distances have demonstrated the drawbacks of these traditional methods with their inherent slowness. Thus, consumer safety and protection is hard to ensure since positive test results are often obtained after the product was put into circulation.

In contrast to the conventional methods, the development of rapid methods allows the fast detection of pathogens in food samples (Dwivedi and Jaykus, 2011; Jasson et al., 2010). Molecular methods, most notably PCR-based technologies or microarrays, have been demonstrated to detect pathogens in a highly specific manner (Malorny et al., 2009). Fluorescence *in situ* hybridization (FISH) represents a promising alternative method in food microbiology among other culture-independent techniques like denaturing gradient gel electrophoresis (DGGE) (Cocolin et al., 2013). Like other rapid techniques, FISH can be performed without relying on the cumbersome and lengthy conventional cultivation and has additional benefits as it visualizes whole cells and targets ribosomal RNAs (or other abundant structures like multi-copy genes), which provides FISH with the capability of distinguishing between viable organisms and dead material (Bottari et al., 2006; Brehm-Stecher, 2008; Jasson et al., 2010). Within a few hours and limited efforts results can be obtained in an enzyme-independent manner and, if desired, also independent of cultivation. Consequently, even viable but nonculturable (VBNC) or, in general, difficult to cultivate pathogens can be identified, which cannot be achieved by the established cultivation procedures. However, FISH is not yet routinely used to analyse and monitor food products. The use of FISH as a valuable and promising tool to address food safety issues depends on its ability to detect pathogens in a highly specific, sensitive and rapid manner. A major challenge for these goals is the crucial influence of the food matrix. In addition, characteristics like low costs per sample, the feasibility of high-throughput-analyses and, ideally, a high degree of simplicity concerning the performance of a test are desirable. These features should, at least, be comparable to recent advances in the use of conventional methods as well as of molecular and other novel tests. Therefore, the purpose of this review is to give an overview of the current state of the art of FISH-testing on diverse kinds of food and to assess the potential of FISH diagnostics with respect to food safety and the detection of foodborne bacteria. Especially the suitability for the implementation into routine testing is of great interest considering the obvious limitations of the currently employed methods.

2. Fluorescence *in situ* hybridization

During the early 1990s, FISH has gained increasing importance as a novel system to detect and identify microorganisms. Amann et al. (1990a) and DeLong et al. (1989) developed a convenient FISH method for the accurate identification of microorganisms in different settings by targeting the highly abundant ribosomal RNAs (rRNA) within bacterial cells (primarily 16S rRNA of the small ribosomal subunit or 23S rRNA of the large ribosomal subunit). Since then, FISH has become a standard method in different biological and medical fields and its establishment has produced significant new scientific insights owing to the substantial progress made in the following years (Amann et al., 1990b, 1995; Amann and Fuchs, 2008; Wagner and Haider, 2012). FISH is routinely used in medicine and diagnostics to rapidly and conveniently identify pathogens in the blood or the faeces and for cytogenetic examinations to detect chromosomal disorders or tumor cells, as well as in ecology and environmental biology to study the composition, growth and changes of complex microbial communities and biofilms (Bottari et al., 2006; Cocolin and Ercolini, 2008; Jehan et al., 2012). Due to the fact that rRNAs possess regions of high variability as well as regions which might be remarkably conserved throughout an entire domain, the differentiation is possible on several taxonomic levels, ranging from distinguishing between related species up to comprising whole kingdoms and domains (Amann and Kühl, 1998).

Although protocols for FISH might differ significantly, the general methodical procedure involves a fixation step of the sample, the permeabilisation to allow the entry of fluorescent probes, the hybridization of the probe to the target sequence, the removal of unbound and excess probes by washing and, finally, the observation of the cells by microscopy or via flow cytometry (Amann and Fuchs, 2008). In food microbiology, additional steps for the sample preparation and homogenization, preenrichment procedures or bacterial separation might be required. In Fig. 1 a flow-chart of FISH-testing of food products including special FISH adaptations is given. Especially the first step regarding food sample pretreatment might differ from FISH tests examining other types of sample material. All steps of a FISH test have been shown to require considerable efforts for optimization. It is, for example, necessary to determine the ideal hybridization time and temperature, to use proper permeabilization and fixation conditions as well as to design highly specific probes (Amann et al., 1995; Wagner et al., 2003). In recent years, free *in silico* modelling software tools have considerably simplified this optimization process (ARB-project: Ludwig et al., 2004; mathFISH: Yilmaz et al., 2011). In case of food and medical microbiology, commercially available FISH kits with

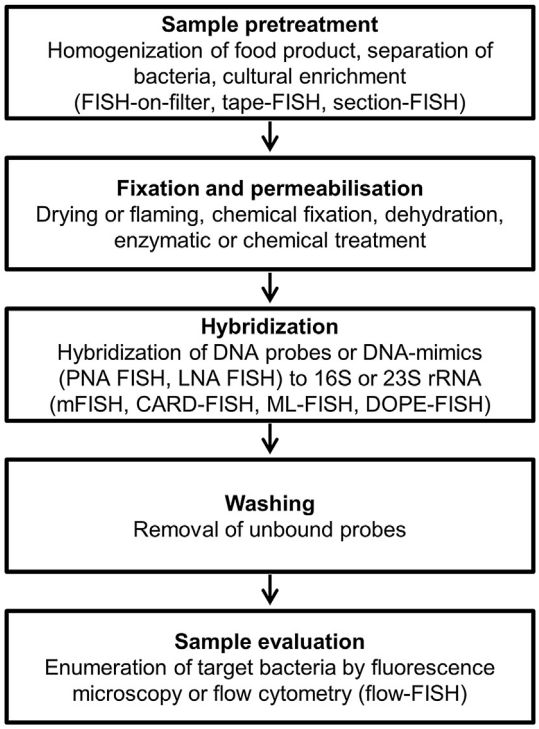

Fig. 1. Schematic representation of a FISH experiment. Overview of the five steps in a FISH experiment including special adaptations with relevance for the use in food microbiology.