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Rapid and specific detection of *Listeria monocytogenes* in smoked salmon with BAX®-PCR

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Abstract

Members of the genus *Listeria* are ubiquitous, and are therefore also common to the food and the environment. Among them, only *Listeria monocytogenes* has a pathogenic potential, and can cause infectious diseases (listeriosis) in humans. Conventional microbiological testing methods are labour-intensive and time consuming (4–5 days), and often require a number of different culture media for final isolation and confirmatory tests. In order to overcome these limitations, numerous rapid methods have been developed in recent years. DNA-based methods such as the polymerase chain reaction (PCR) have increasingly been used for rapid and sensitive detection of *L. monocytogenes*. Among the various available PCR assays, we used the BAX® system (with two different detection procedures: gel detection and automated detection) to screen for *L. monocytogenes* in samples of vacuum packaged cold smoked salmon. A total of 27 samples were used for this study. The method was compared to the German standard microbiological detection method according to DIN 11290-1 and -2. Detection of *Listeria* and *L. monocytogenes* from salmon samples was performed using Palcam enrichment medium, followed by plating on both Palcam agar and ALOA agar. The BAX® assay gave identical results for 26 food samples compared to the standard method, including 15 positives. Only in one case the BAX® system gave a false-positive result, probably due to the amplification of DNA from nonviable cells of *L. monocytogenes*. In naturally contaminated food samples, the BAX® method gave good results after 24–48 h. Application of this rapid method is simple and time saving.

Keywords: *Listeria monocytogenes*; PCR; BAX® system

1. Introduction

Consumers around the world are more aware than ever about food safety issues and are seeking increased assurance about safety and quality of the foods they eat. The cost of illness, death, and business lost due to bacterial foodborne diseases is high. Some pathogenic bacteria that cause economically important foodborne diseases in the United States include *Salmonella* (calculated at $4 billion in economic losses annually), *S. aureus* ($1500 million) and *Listeria monocytogenes* ($313 million) (Todd, 1989). In 2000, the Federal Institute of Health Protection of Consumers and Veterinary Medicine in Germany reported overall 200,000 cases of foodborne infections. Studies by this institute showed in some cases that illnesses caused by zoonotic pathogens had increased markedly compared to 1999. *L. monocytogenes* was repeatedly found in samples of meat and meat products, in raw milk, soft cheese and pasteurised dairy products and especially in fish and fish products. Several surveys reported the presence of the pathogen *L. monocytogenes* in cold smoked salmon (Ben Embarek, 1994;

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Dillon, Patel, & Ratnam, 1994; Norton et al., 2001). High counts of *L. monocytogenes* of more than 10,000 per gram of food were detected in some cases (Feldhusen, Jark, Etzel, Ballin, & Wilke, 2002). According to the latest scientific findings, a contamination level of higher than 100 *Listeria* per gram may cause illness. Contamination by levels exceeding 100 viable cells of *L. monocytogenes* per gram food are therefore considered to have the potential of causing serious, sometimes fatal, foodborne infections in young children, the elderly and people with weakened immune systems. It also can cause miscarriages or stillbirths even if a pregnant woman experiences no symptoms. In healthy people, *L. monocytogenes* can cause headache, high fever, nausea, abdominal pain and diarrhoea. Zero-tolerance for *L. monocytogenes* was introduced in the USA for commercial foods for consumption without further cooking. Other countries such as Switzerland, Austria, Italy, New Zealand and South Korea have adopted this regulation. Still, *Listeria* cannot be eliminated completely from food and food processing lines in the industry. Therefore, a tolerance of up to 100 *L. monocytogenes* per gram of food on the "sell-by" date would be more realistic. Considerations leading to this "tolerance" level probably have accepted this as "minimal infection dose" for people at risk. The mortality rate of 30% for listeriosis is the highest of any foodborne bacterial agent. This value is an average rate and will be higher for infants and people with immune deficiencies. Finding a quick and reliable method to detect and identify *L. monocytogenes*, has high priority for the early recognition of contaminated food products. The modern polymerase chain reaction (PCR) based assays provide a quick and reliable screening procedure for several foodborne pathogens (Baumgartner & Grand, 1995; Hoffman & Wiedmann, 2001; Lücke & ten Bosch, 1998; Winters, Maloney, & Johnson, 1999). One of the latest test kit available for the rapid detection of *L. monocytogenes* is the BAX® system (Du Pont Qualicon, Germany). In April, 2002, the US Department of Agriculture's Food Safety and Inspection (FSIS) has adopted the BAX® system to screen meat and poultry samples for *L. monocytogenes* (*FSIS adopts new screening method for Listeria monocytogenes*, 2002). The scope of this study was to critically evaluate the use of this system for screening *L. monocytogenes* in cold smoked salmon.

2. Materials and methods

A total of 27 samples of vacuum packaged smoked salmon (obtained from local supermarkets) were screened for *L. monocytogenes* using cultural *Listeria* detection according to DIN 11290-1 and -2 with a small modification, and the molecular based methods, automated BAX® and gel-based system.

2.1. Listeria—culture procedure

A 25 g portion of each sample was blended in a stomacher in 225 ml of Palcam-Listeria-enrichment-broth (Merck, Germany) for 2 min and incubated at 37 °C for either 24 or 48 h. The enrichment method using Palcam broth is not recommended for the BAX® system, but since this medium is often used in Germany for routine isolation purposes it was included in this study. For determination of viable *Listeria* counts, a loopful from each enrichment was surface-plated, both on Palcam-*Listeria*-Agar (Merck, Germany) and the new selective ALOA agar (AES-Laboratoire, France). The plates were incubated for 24–48 h at 37 °C. Five suspect *Listeria* colonies from each plate were chosen and purified on Standard-I-Agar. Further biochemical characterisation was performed using API-Listeria (bioMérieux, Germany) as well as other tests like catalase, oxidase and CAMP reactions (with *Staphylococcus aureus* DSM 6732 and *Rhodococcus equi* DSM 20307).

2.2. PCR

Subsequently to the enrichment procedure, the BAX® system protocol (Du Pont Qualicon, Germany) was followed.

2.3. BAX® system with gel detection

The process consists of three major steps:

- DNA preparation

One ml of enriched samples were centrifuged at 13,000*g* for 10 min. The pellet was washed twice in sterile demineralised water. Afterwards, 5 μl of the cell suspension was recovered in 200 μl lysis reagent (12.5 μl protease to 1 ml lysis buffer) and heated at 55 °C for 60 min, then 95 °C for 10 min. After cooling (about 5 min) 50 μl of lysed samples from lysis tubes were transferred into the PCR tubes.

- Amplification

BAX® system tablets contain all necessary reagents for PCR amplification (buffer, primers, polymerase, dNTPs and positive control). The PCR tubes containing tablets and lysate were placed in the thermal cycler (Primus 25, MWG-Biotech, Germany), and PCR was carried out using the following condition: denaturation step at 94 °C for 2 min, followed by 38 amplification cycles. Each cycle consisted of 15 s at 94 °C and 3 min at 70 °C. Negative and positive controls were included in all experiments.

- Detection

15 μl of PCR products were loaded onto 2% agarose gels and electrophoresis was performed for 45 min at 180 V. The molecular weight marker supplied with the kit was also run on each gel. The gels were stained with