Comparison of two pre-enrichments broths for recovering *Listeria* spp. from salmon (*Salmo salar)* and salmon-trout (*Oncorhynchus mykiss)*

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Abstract

Low levels of occurrence of *Listeria* spp. in fresh salmon (*Salmo_salar*) and salmon-trout (*Oncorhynchus mykiss*), may be related to the selectivity of the pre-enrichment broth recommended by ISO 11290-1. The purpose of this study was to compare the abilities of Fraser base (without supplements) and 0.1% (w/v) peptone water for recovering *Listeria* spp. from the fresh fish samples.

Fifty-six fish were swabbed and the swabs placed in Fraser base and in 0.1% (w/v) peptone water. Samples were analysed 4–6 h later following the ISO 11290-1 protocol. A total of fifteen *Listeria* spp. positive samples were found. Three and twelve samples were found to contain respectively, *L. monocytogenes* and *L. innocua*. The Fraser base did not detect any of the three *L. monocytogenes* positive samples. Only two *Listeria* spp. positive samples were simultaneously recovered by the two broths.

1. Introduction

Listeria monocytogenes in foods can pose a significant health risk, with a relatively high mortality for specific sections of the population, such as pregnant women, foetuses and immunocompromised people.

Listeria spp. have been found in raw fish of fresh water and marine origins and *L. monocytogenes* was isolated from 62% of all water samples (Dillon & Patel, 1992). Farber (1991) reported the presence of *L. monocytogenes* in raw salmon from USA, Chile, Norway and Canada. *L. monocytogenes* was isolated from thawing water, from raw salmon skin, from filleting tables, from rinsing water and from product trimmings of a cold-smoking plant. They concluded that the initial source of contamination was the raw fish (Eklund et al., 1995). This conclusion was in agreement with an investigation on three salmon-trout smoking plants in Switzerland where it was shown that the raw fish, in particular, was more contaminated with *L. monocytogenes* than the finished products (Jemmi & Keusch, 1994). Currently, in Europe and North America, fish smoking is used simply as a means of imparting a desirable flavour and aroma, and today's salting and smoking steps are much lighter. In the cold-smoking process (temperature of the smoke below 30°C) the finished product does not undergo any listericidal steps and could be a source of *L. monocytogenes*.

As reported by several authors, examining food for the presence of *Listeria* spp. requires a pre-enrichment step because of their susceptibility to injury and consequent inability to grow in selective media.

The distance between the fish smoking factories and the laboratory, when the occurrence of *Listeria* spp. in Portuguese cold-smoked fish products was ascertained (Duarte, Vaz-Velho, & Gibbs, 1995), made necessary a choice between the use of half-strength Fraser broth, as the International Standard ISO 11290-1 (1996) suggests, and the use of a non-selective broth medium, since the collected samples had to be maintained in the broth for up to one week before the analysis was begun. Due to the possibility of sub-lethal injury of *Listeria* spp. by the

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use of the ISO 11290-1 method, peptone water was used instead of Fraser base with half of the additives. However, at that time, no experiments were done in order to ascertain which pre-enrichment method performed better and the role of competitive flora might have been underestimated by using peptone water for such a long period.

Taking into account the low levels of occurrence of *Listeria* spp. found in the raw material for cold-smoking processes (Vaz-Velho, Duarte, & Gibbs, 1998), we decided to compare Fraser base and 0.1% (w/v) peptone water as pre-enrichment procedures for the recovery of *Listeria* spp. from fresh salmon (*Salmo salar*) and salmon-trout (*Oncorhynchus mykiss*) samples.

2. Materials and methods

2.1. Sampling procedure

Fifty-six fish samples (20 of Norwegian farmed salmon and 36 of Portuguese farmed salmon-trout) were analysed. Fishes were packed in polystyrene boxes. Five fishes from each box were considered as one sample. Ten square centimetres of the fresh fish skin were swabbed. A pair of swabs was used simultaneously for each fish, and each swab was placed in bottles with either 25 ml of sterile 0.1% (w/v) peptone water (LabM, MC5, Bury, UK) + 5 g/l of NaCl (Merck, 6404, Darmstadt, Germany) (5 swabs per sample), or 250 ml of Fraser broth base (Merck 110398.0500) with no selective suplement added (5 swabs per sample). The samples were transported to the laboratory inside chilled portable insulated boxes, placed at room temperature and analysed 4–6 h later following the ISO 11290-1 protocol as described below.

2.2. Isolation procedure

Swabs of the fresh fish in 25 ml of peptone water were transferred to 225 ml of Fraser primary enrichment-half-strength Fraser broth (Merck 110398.0500) which contains half of the concentration of the selective supplement (Merck 110399.0001)-compared to Fraser broth. Fraser selective supplement (one vial) was added to the 250 ml of Fraser base. All samples were homogenised and incubated at 30°C for 24 h.

0.1 ml of these primary enrichments were transferred to 10 ml of secondary enrichment Fraser broth (half-strength Fraser plus another vial of Fraser supplement) and incubated at 35°C for 24–48 h. Therefore Fraser secondary enrichment contains 25 mg/l of acriflavine and 500 mg/l of ammonium iron(III) citrate. All the enrichments, whether showing growth or not, were subcultured by streaking onto Oxford (Merck, 107004.0500) and PALCAM (Merck, 11755.0500) selective agars (both incubated at 30°C, 48 h). Typical colonies (five per plate) were streaked on tryptone soy yeast extract agar (tryptone soy broth (Lab M LAB4) + 6 g/l yeast extract (Lab M, MC1) + 12 g/l agar (Lab M, MC2)) and incubated at 37°C for 24 h.

2.3. Confirmation and identification procedures

All the isolates were confirmed to the genus level by Gram, catalase and oxidase tests, and tumbling motility (tryptone soy broth, 25°C, 24 h), and to the species level by API *Listeria* (BioMérieux, 10300) and by the Christie–Atkins–Munch–Peterson (CAMP) test with *Staphylococcus aureus* ATCC 25923 (Food Quality Centre, Escola Superior de Biotecnologia, Porto, Portugal) and *Rhodococcus equi* NCTC 1691 (Leatherhead Food Research Association, Surrey, UK) on sheep blood agar plates (BioMérieux, 43041).

3. Results and discussion

Fifty-six fresh fish samples (20 of Norwegian ocean reared salmon and 36 of Portuguese farmed salmon-trout) were analysed. *Listeria* spp. were isolated from 15 samples. 3 and 12 samples were found to contain respectively, *L. monocytogenes* and *L. innocua* (Table 1).

Although Fraser base could recover *L. innocua*, it failed to recover the three *L. monocytogenes* positive samples. *L. monocytogenes* was found alone in these samples but, as reported by Curiale and Lewus (1994), *L. innocua* has a shorter generation time than *L. monocytogenes* and that recovery of *L. monocytogenes* from

Table 1
Recovery of *L. innocua* and *L. monocytogenes* from fresh salmon and salmon-trout samples using 0.1% (w/v) peptone water and Fraser base as pre-enrichment broths

Enrichment broth	*Listeria* spp.	*L. monocytogenes*	*L. innocua*
Only peptone water	8/15	3/3	5/12
Only Fraser base	5/15	0/3	5/12
Both broths	2/15	0/3	2/12
Total of positives	15	3	12

foods using selective broths was lower when *L. innocua* was present.

Thurette (1995), when comparing the ISO protocol using Fraser base with or without additives as a pre-enrichment broths for resuscitating *Listeria* spp. from smoked salmon, concluded that the resuscitation step increased the number of positive samples, and that those numbers increased with the resuscitation time, 6 h performing better than 4 h.

Budu-Amoako, Toora, Walton, Ablett, and Smith (1992), using the non-selective broth tryptone soy broth with yeast extract and the *Listeria* Enrichment broth with selective agents, found the non-selective broth to be better for the recovery of stressed *L. monocytogenes*.

Beumer, Giffel, Anthonie, and Cox (1996) stated that enrichment broths having lower acriflavine concentrations and an adequate buffer favoured the isolation of *L. monocytogenes*.

Fraser base (no added suplements), despite the absence of acriflavine, compared to the half-strength Fraser of the ISO protocol, still has other selective agents such as nalidixic acid (20 mg/l) and lithium chloride (3 g/l). It also contains four times the amount of salt of the peptone water used in this study (20 g/l rather than 5 g/l). Patel and Beuchat (1995) reported that Fraser broth showed lower recoveries of heat-injured *Listeria* spp., probably due to the presence of lithium chloride.

Despite the low levels of lithium chloride in Fraser base, its presence together with the other selective agents may have inhibited the growth of *L. monocytogenes*. Even for these raw fish samples not subjected to any stressful processing factors, the use of a non-selective pre-enrichment broth such as peptone water clearly improved the recovery of *L. monocytogenes*. Thus, these findings confirm the advisability of incubating the swabs for a short period of time (4–6 h) in a broth without selective agents to improve the detection of *Listeria* spp. from this type of material.

Although *Listeria* spp. were found in 15 samples only two samples were simultaneously positive by both protocols. In this experiment, the use of two different pre-enrichment broths increased the recovery of *Listeria* spp. If just one protocol was used, five (only peptone water) or eight (only Fraser base) fewer positive samples would have been found.

These differences between methods underline the probability of underestimation of the occurrence of *L. monocytogenes* when just one protocol is used for its detection. Rather than promoting a single method for detection of *Listeria* spp. and *L. monocytogenes*, the benefit of using a combination of methods should be emphasised.

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References

Beumer, R. R., Giffel, M. C., Anthonie, S. V. R. ., & Cox, L. J. (1996). The effect of acriflavine and nalidixic acid on the growth of *Listeria* spp. in enrichment media. *Food Microbiology*, *7*, 311–325.

Budu-Amoako, E., Toora, S., Walton, C., Ablett, R. F., & Smith, J. (1992). Thermal death times for *Listeria monocytogenes* in lobster meat. *Journal of Food Protection*, *55*(3), 211–213.

Curiale, M., & Lewus, C. (1994). Detection of *Listeria monocytogenes* in samples containing *Listeria innocua*. *Journal of Food Protection*, *57*, 1048–1051.

Dillon, R., & Patel, T. (1992). *Listeria* in seafoods: a review. *Journal of Food Protection*, *55*(12), 1009–1015.

Duarte, G., Vaz-Velho, M., & Gibbs, P. (1995). Occurrence of *Listeria* spp. and *Listeria monocytogene*s in Portuguese cold-smoked fish products. *Arquivos do INSA*, *20–21*, 55–61.

Eklund, M. W., Poysky, F. T., Paranjpye, R. N., Lashbrook, L. C., Peterson, M. E., & Pelroy, G. A. (1995). Incidence and sources of *Listeria monocytogenes* in cold-smoked fishery products and processing plants. *Journal of Food Protection*, *58*(5), 502–508.

Farber, J. (1991). *Listeria monocytogenes* in fish products. Journal of Food Protection 54(12), 922–924, 934.

International Standard ISO 11290-1 (1996) Microbiology of food and animal feeding stuffs – Horizontal method for the detection and enumeration of *Listeria monocytogenes*. Technical Commitee ISO/TC 34, Agricultural food products, Subcommittee SC 9, Microbiology.

Jemmi, T., & Keusch, A. (1994). Occurrence of *Listeria monocytogenes* in freshwater fish farms and fish-smoking plants. *Food Microbiology*, *11*, 309–316.

Patel, J., & Beuchat, L. (1995). Evaluation of enrichment broths for their ability to recover heat-injured *Listeria monocytogenes*. *Journal of Applied Bacteriology*, *78*, 366–372.

Thurette, J. (1995). *Listeria and smoked salmon: purchase data for predictive microbiology*. Ph.D. Thesis, University of Lille, France.

Vaz-Velho, M., Duarte, G., & Gibbs, P. (1998). Occurrence of *Listeria* spp. in salmon-trout (*Oncorhynchus mykiss*) and salmon (*Salmo salar*). *Food Science and Technology International*, *4*(2), 121–125.