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Original article

Monitoring of bacterial evolution and molecular identification of lactic acid bacteria in smoked salmon during storage

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Abstract – The microflora of cold smoked salmon (CSS) consists of Gram-negative bacteria such as *Photobacterium, Vibrio* and *Shewanella*, and Gram-positive bacteria from the genera *Carnobacterium*, *Lactobacillus* and *Brochothrix*. In this work, 5 series of CSS samples from different suppliers were stored for 1 week at 4 °C and 4 weeks at 7 °C. The enumeration of lactic acid bacteria (LAB) from each sample was carried out every week followed by isolation. Molecular identification was carried out on isolates obtained from 15 CSS samples stored at refrigeration temperature for 14 to 35 d. 158 Gram-positive bacteria were identified by PCR using specific primers and PCR-RFLP on the 16S-23S rDNA intergenic spacer region (ISR). 28 *Carnobacterium piscicola* strains, 15 *Cb. divergens* strains, 81 *Lactobacillus curvatus* strains and only 1 *Lb. sakei* strain were identified. In addition, 33 Gram-positive catalase-positive isolates were identified as *Brochothrix thermosphacta*. The modification of bacterial diversity in CSS during storage was also monitored by using the temporal temperature gradient gel electrophoresis (TTGE) technique. The migration allowed both Gram-positive bacteria (*Cb. piscicola*, *Cb. divergens*, *Lb. curvatus*, *Lb. sakei* and *B. thermosphacta*) and Gram-negative bacteria (*P. phosphoreum* and *S. putrefaciens*) to be differentiated. All the bacteria species (*Cb. piscicola*, *Lb. curvatus* and *P. phosphoreum*) identified after culture could be specifically detected by TTGE.

Smoked salmon / lactic acid bacteria / 16S-23S rDNA intergenic spacer region / TTGE

Résumé – Étude de l'évolution bactérienne et identification moléculaire des bactéries lactiques du saumon fumé au cours de sa conservation. La flore du saumon fumé est constituée d'une flore Gram négatif composée principalement des genres *Photobacterium*, *Vibrio* et *Shewanella* et de bactéries à Gram positif des genres *Carnobacterium*, *Lactobacillus* et *Brochothrix*. Cette recherche a concerné 5 séries d'échantillons de saumon fumé de différentes usines conservées 1 semaine à 4 °C et 4 semaines à 7 °C. Le dénombrement des bactéries lactiques a été réalisé chaque semaine sur chaque échantillon suivi par des isolements. L'identification moléculaire spécifique de ces isolats a été conduite sur 15 échantillons conservés pendant une durée de 14 à 35 j. 158 isolats de bactéries Gram positif ont été identifiés en utilisant amorces spécifiques et PCR-RFLP sur la séquence intergénique 16S-23S rDNA. Ce travail a donc permis d'identifier 28 isolats de *Carnobacterium piscicola*, 15 isolats de *Cb. divergens*, 81 isolats de *Lactobacillus curvatus* et un

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isolat de *Lb. sakei*. De plus, 33 isolats Gram positif catalase positif ont été identifiés comme étant des souches de *Brochothrix thermosphacta*. Le changement de la diversité bactérienne dans le saumon fumé pendant le stockage a été suivi par la méthode TTGE (temporal temperature gradient gel electrophoresis). La migration des bactéries de références a permis de différencier les bactéries Gram positif (*Cb. piscicola*, *Cb. divergens, Lb. curvatus, Lb. sakei* et *B. thermosphacta)* mais aussi les bactéries Gram négatif (*P. phosphoreum* et *S. putrefaciens*). Toutes les bactéries (*Cb. piscicola*, *Lb. curvatus* et *P. phosphoreum*) identifiées par culture peuvent aussi être détectées par TTGE.

Saumon fumé / bactérie lactique / région intergénique 16S-23S rDNA / TTGE

1. INTRODUCTION

The microflora of cold smoked salmon (CSS) consists of Gram-positive bacteria dominated by LAB [12, 23] and Gram-negative bacteria such as *Shewanella putrefaciens* and *Photobacterium phosphoreum,* which could play an alteration role [4, 7]. The population of Gram-positive bacteria is enhanced during storage [17]. C*arnobacterium piscicola* and species of *Lactobacillus* have been identified as predominant flora in CSS stored at 2 °C for 3 weeks. *Cb. piscicola* dominates at the beginning of the storage while lactobacilli such as *Lb. sakei*, *Lb. curvatus, Lb. farciminis* and *Lb. alimentarius* grow later [6, 12, 23]. *Brochothrix thermosphacta*, one of the other Gram-positive bacteria isolated and *Lactobacillus* species such as *L. sakei* are involved in off-flavour production, modifying the organoleptic qualities of CSS [17].

Identification of bacteria using traditional methods is time-consuming and may lead to false identification. Molecular biology methods are useful for analysing microbial ecology. Recently, the bacterial diversity of CSS was approached using amplification of rDNA restriction analysis (ARDRA), a culture-independent method [3]. This method allowed the detection of *Vibrio* species, *Enterobacteriaceae* and also marine psychrophilic bacteria related to *Alteromonas macleodii*, which was not isolated from the cultures. However, no Gram-positive species were detected using ARDRA. In spoiled 'gravad' rainbow trout, *Lb. sakei*, *Lb. curvatus* and *Cb. piscicola* were the major LAB species identified using ribotyping and associated with the spoilage of this product [14].

PCR based on the 16S-23S rDNA intergenic spacer region (ISR) for rapid bacterial identification has been developed [8]. Species-specific primers useful for PCR identification of *Lactobacillus* species were designed from this region [2, 19–21]. Length polymorphism of the 16S-23S rDNA ISR and RFLP technique allowed the differentiation of closely-related *Carnobacterium* [10]. Recently, the modification of bacterial diversity has been monitored by using denaturing gradient gel electrophoresis (DGGE) or temporal temperature gradient gel electrophoresis (TTGE) [15]. The TTGE technique has been used successfully to identify the bacterial diversity of cheeses and other complex ecosystems [9, 16, 25].

In this study, the LAB microflora of CSS was identified by using a PCR-based method on 16S-23S rDNA ISR. TTGE was evaluated in order to monitor the evolution of the bacterial population during storage of this product.

2. MATERIALS AND METHODS

2.1. CSS samples and bacterial strains

The smoked salmon samples furnished by 4 French smokehouses and bacterial strains used in this study are listed in Table I and Table II, respectively. CSS samples were stored at 4 °C for 1 week then at 7 °C for 4 weeks.

Table I. Smoked salmon samples analysed.

Smokehouse	Origin of salmon
A	Norway, cultivated salmon
B	Norway (B1); Ireland (B2)
C	Scotland
D	Norway

Table II. Bacterial strains used in this study.

Species	Strain designation
Carnobacterium piscicola	NCDO 2762
Carnobacterium divergens	NCDO 2763
Lactobacillus sakei	ATCC 15521
Lactobacillus curvatus	DSM 20019
Brochothrix thermosphacta	DSM 20171
Photobacterium phosphoreum	CIP 102511
Shewanella putrefaciens	CIP 6929
Escherichia coli	JM109

2.2. LAB enumeration and isolation

Forty grams of CSS were homogenised in a stomacher in 160 mL sterile tryptone salt water. A 1-mL aliquot of the homogenised samples was serial diluted in sterile tryptone salt water and 0.1 mL of each appropriate dilution was spread onto Elliker (Biokar, Beauvais, France) agar plates. Enumeration was carried out after 48 h of incubation at 30 °C. Strains isolated as pure cultures were stored at –80 °C. The genus *Brochothrix* can be distinguished from *Listeria* by a motility test at 25 °C and no growth at 35 °C [18]. The API 50CHL-gallery test was used to identify Gram- and catalase-positive isolates.

2.3. Bacterial DNA extraction

Total DNA from type strains and Gram-positive catalase-negative isolates was extracted as previously described [24].

Bacterial DNA extraction from CSS samples was carried out as previously described [5] with some modifications: 10 g of CSS were homogenised using a stomacher in 10 mL of 4 mol·L^{-1} guanidine thiocyanate 0.1 mol·L^{-1} Tris (pH 7.5) and 150 µL 10% N-lauroyl sarcosine-0.1 mol·L^{-1} phosphate buffer (pH 8.0). 250 µL of the homogenate was transferred into a 2-mL polypropylene microtube and the remainder was frozen. After the addition of 500 µL of 5% N-lauroyl sarcosine-0.1 mol·L^{-1} phosphate buffer (pH 8.0), the 2-mL tube was incubated at 70 °C for 1 h. One volume (750 µL) of 0.1-mm-diameter silica beads (Sigma-Aldrich, Saint-Quentin-Fallavier, France) previously sterilised by autoclaving was added, then the tube was shaken in a Bead-Beater® (BioSpec Products Inc., Bartlesville, OK, USA) twice for 5 min. After addition of polyvinylpolypyrrolidone (15 mg), the tube was vortexed and centrifuged for 5 min at 12 000 × *g*. After recovery of the supernatant, the pellet was washed with 500 µL of TENP buffer (50 mmol·L^{-1} Tris (pH 8.0), 20 mmol·L^{-1} EDTA (pH 8.0), 100 mmol·L^{-1} NaCl, 1% polyvinylpolypyrrolidone) and centrifuged for 5 min at 12 000 × *g*. The resulting supernatant was added to that previously obtained. This washing step was repeated three times. The pooled supernatants (about 2 mL) were briefly centrifuged to remove particles and then split into 2-mL tubes. Nucleic acid precipitation was performed as previously described [5].

2.4. PCR reactions

The oligonucleotide primers (Invitrogen Life Technology, Cergy-Pontoise, France) used in this study are listed in Table III. ISR amplification using 16S/2 and 23S/7 primers was performed in order to identify LAB genera. Specific primers for *Lb. sakei*, *Lb. curvatus* and *Lb. plantarum* were used to determine *Lactobacillus* species. tAla-23S/10 primers were used to amplify *Carnobacterium* rDNA. Bacterial DNA from CSS samples was amplified using FG984GC and L1401 primers [9, 25]. PCR reactions were performed in a total volume

Table III. Primers used in this study.

Primer	Location	Oligonucleotide sequence (5'→3')	Annealing temperature °C	Reference
16S/2	16S rRNA gene, forward (position 1390-1407)	CTTGTACACACCGCCCGTC	56	[8]
23S/7	23S rRNA gene, reversed (position 188-208)	GGTACTTAGATGTTTCAGTTC	56	[8]
23S/10	23S rRNA gene, reversed (position 456-474)	CCTTTCCCTCACGGTACTG	58	[8]
tAla	tRNA ^{Ala} gene, forward	TAGCTCAGCTGGGAGAGC	58	[11]
Lc	*Lb. curvatus* 16S/23S rRNA ISR gene, reversed	TTGGTACTATTTAATTCTTAG	53	[2]
Ls	*Lb. sakei* 16S/23S rRNA ISR gene, reversed	ATGAAACTATTAAATTGGTAC	53	[2]
Lpl	*Lb. plantarum* 16S/23S rRNA ISR gene, reversed	ATGAGGTATTCAACTTATG	53	[2]
FG984GC	V6-V8 region of 16S rDNA, forward	CGCCCGGGGCGCGCCCCGG GCGGGGCGGGGCACGGGGG GAACGCGAAGAACCTTAC	60	[9]
L1401	V6-V8 region of 16S rDNA, reversed	GCGTGTGTACAAGACCC	60	[25]

of 25 µL containing 1% µg·mL^{-1}, 1X PCR buffer, 2.5 mmol·L^{-1} $MgCl_2$, 0.2 µmol·L^{-1} of each primer, 0.2 mmol·L^{-1} (each) dNTP and 1U *Taq* DNA polymerase (BioLabs, Hitchin, UK). The amplification programme consisted in a 1-min denaturation step at 94 °C, a 1-min annealing step (at the optimal annealing temperature for each primer listed in Tab. III) and a 1-min extension step at 72 °C. The first cycle was preceded by incubation for 5-min at 94 °C. After 35 cycles, there was a final 5-min extension at 72 °C. Negative controls containing no DNA template were included in parallel. PCR products were separated in a 1.5% (w/v) agarose gel and were subsequently visualised by UV illumination after ethidium bromide staining.

2.5. Restriction enzyme analysis

In order to identify *Carnobacterium* species isolated from CSS, the tAla-23S/10 amplified PCR products were subjected to digestion with *Hin*d III (BioLabs) [10]. Digestion was performed in a 25-µL mixture at optimal conditions according to the manufacturer's protocols. The total digested products were separated by electrophoresis in a 2% (w/v) agarose gel.

2.6. TTGE analysis

PCR products obtained by amplification using primers targeting the V6-V8 region of 16S rDNA were subjected to TTGE analysis. TTGE was performed by using the DCode Universal Mutation Detection System (BioRad, Marnes-la-Coquette, France) and gels of 16 cm by 16 cm by 1 mm. Gels were prepared with 11% (w/v) acrylamide stock solutions (acrylamide-bisacrylamide; 37.5:1) and a final urea concentration of 9.7 mol·L^{-1}. Gels were run with 1.25X TAE buffer. TTGE parameters and gradient temperatures were optimised

Table IV. Enumeration of Gram-positive bacteria on Elliker agar plates from CSS samples.

Sample	Gram-positive bacteria (cfu·g^{-1})						
	Fresh salmon	After smoking salmon	J0	J14	J21	J28	J35
Salmon A	2.2×10^2	20	1.4×10^2	1.1×10^5	10^6	7.1×10^5	3×10^6
Salmon B1	3×10^2	25	3×10^2	1.2×10^4	8×10^5	4.2×10^6	7.2×10^6
Salmon B2	1.2×10^2	2.8×10^2	27.5	9.2×10^4	3.8×10^5	1.9×10^7	1.2×10^8
Salmon C	7.8×10^3	20	80	3.6×10^6	1.1×10^7	1.7×10^7	5.8×10^7
Salmon D	ND	ND	3.4×10^2	10^6	2.5×10^6	2.9×10^7	3×10^6

to separate the bacterial species studied. The final electrophoresis conditions were 55 V for 18 h with an initial temperature of 65.5 °C and a final temperature of 69.5 °C (the temperature was increased by 0.2 °C per h). 10-µL samples of PCR products were deposited in wells. To avoid non-homogenous temperature effects, samples were not deposited in the outermost wells. After running, gels were stained for 15 min with SYBR Green I 1X (Molecular Probes, Eugene, OR, USA) and visualised under UV.

2.7. Cloning and sequencing of TTGE separated fragments

Fragments were excised from the TTGE gels and incubated overnight at –20 °C in water. The rDNA was reamplified with FG984GC and L1401 primers then subjected to TTGE analysis to confirm their relative positions. The fragments amplified with FG984 (lacking the GC clamp) and L1401 primers were cloned by using the QIAGEN PCR cloning Kit (Qiagen S.A., Courtabœuf, France) and transformed into *E. coli* JM109. The cloned fragments were sequenced (Genome Express, Meylan, France). DNA sequences were analysed and compared with sequences in the GenBank database using the BLAST software available at NCBI [1].

3. RESULTS

3.1. Enumeration of Gram-positive bacteria

The Gram-positive microflora of five CSS furnished by 4 smokehouses was enumerated during five weeks of storage (Tab. IV). The enumeration of the Norwich CSS (A and B1), showed an increase in Gram-positive bacteria from 10^2 cfu·g^{-1} at day 0 to 10^6 cfu·g^{-1} at day 35. The enumeration of the Irish CSS (B2), showed that Gram-positive bacteria increased from 27.5 cfu·g^{-1} at day 0 to 10^8 cfu·g^{-1} at day 35. Bacterial enumeration of the Scottish sample (C), showed an increase of 5.5 logs of Gram-positive bacteria from day 0 to day 35. In Norwich CSS from smokehouse D, the Gram-positive bacteria grew from 10^2 cfu·g^{-1} at day 0 to 10^6 cfu·g^{-1} at day 35. The results showed generally that Gram-positive bacteria which were at 10^2 to 10^3 cfu·g^{-1} in fresh salmon decreased to 20 to 10^2 cfu·g^{-1} after the smoking process then increased by 4 to 6 logs during five weeks of storage.

3.2. Identification of LAB isolates

158 Gram-positive bacteria were isolated from enumeration agar plates. The 125 catalase-negative isolates were identified as LAB. The genus of these isolates

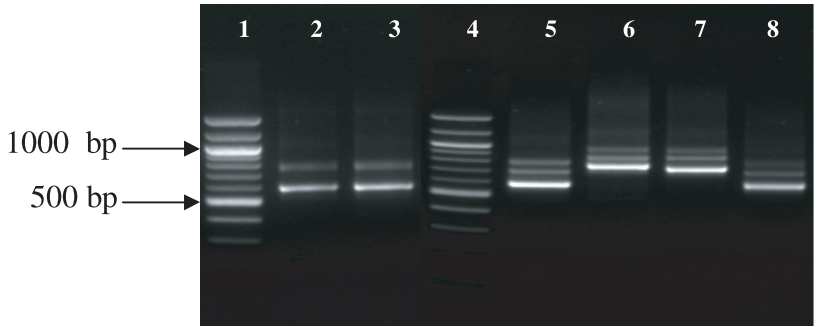

Figure 1. Differentiation of the LAB genera (*Lactobacillus* or *Carnobacterium*) by PCR amplification of the 16S-23S rDNA ISR by 16S/2-23S/7 primers (Lanes 1 and 4: 100-bp molecular weight marker; lane 2: *Lb. curvatus* DSM 20019; lane 5: *Cb. divergens* NCDO 2763; lane 6: *Cb. piscicola* NCDO 2762; lanes 3, 7 and 8: LAB isolates from CSS samples).

was determined by using PCR amplification targeting 16S-23S rDNA ISR. *Lactobacillus* and *Carnobacterium* can be differentiated by their ISR profiles (Fig. 1). *Lactobacillus* has a two-fragment profile while *Carnobacterium* has a three-fragment profile [10]. 43 isolates showing the three-fragment profile belong to the *Carnobacterium* genus.

Identification of *Carnobacterium* species was performed by using the RFLP method based on 16S-23S rDNA ISR described by Kabadjova et al. [10]. 28 isolates showed a profile of two fragments at 460 and 408 bp, which is the profile of *Cb. piscicola*, and 15 isolates showed a profile of two fragments at 422 and 308 bp, which corresponds to the profile of *Cb. divergens* (data not shown). All of the other 82 isolates were identified as *Lactobacillus*. *Lactobacillus* species frequently found in CSS were identified by PCR using *Lb. sakei*, *Lb. curvatus* and *Lb. plantarum* species-specific primers designed from 16S-23S rDNA ISR [2, 12, 22]. 81 strains were identified as *Lb. curvatus* and only one strain of *Lb. sakei* was isolated from the Norwich CSS. No *Lb. plantarum* was detected.

The 33 Gram-positive isolates that produce catalase were also identified. *Brochothrix* and *Listeria,* which are Gram-positive catalase-positive bacteria mainly isolated from CSS, were identified using some specific tests. *B. thermosphacta* was distinguished from *Listeria* because it does not grow at 35 °C and is immobile at 25 °C. All the isolates were identified as *B. thermosphacta* using the API 50CHL-gallery test.

In three CSS samples (A, B1 and B2), the Gram-positive colonies isolated at day 28 and day 35 were identified as *Lb. curvatus* (70 to 100%) and *B. thermosphacta* (10 to 30%). In contrast, after four weeks of storage, *Cb. piscicola* represented 92% of the Gram-positive population in the Norwich CSS (sample D). In the Scottish CSS (sample C), *Lb. curvatus* represented 100% of the population at day 14, while at day 28 *B. thermosphacta* was predominant (72%), followed by *Cb. divergens* (18%) and *Lb. curvatus* (9%).

3.3. TTGE analysis

TTGE analysis was evaluated to monitor the evolution of Gram-positive and Gram-negative bacteria in CSS. The V6-V8 region of 16S rDNA was amplified using extracted bacterial DNA and the primers FG984GC and L1401. The PCR products obtained (approx. 500 bp) were then deposited in TTGE gel. As shown in Figure 2, seven bacterial species were chosen

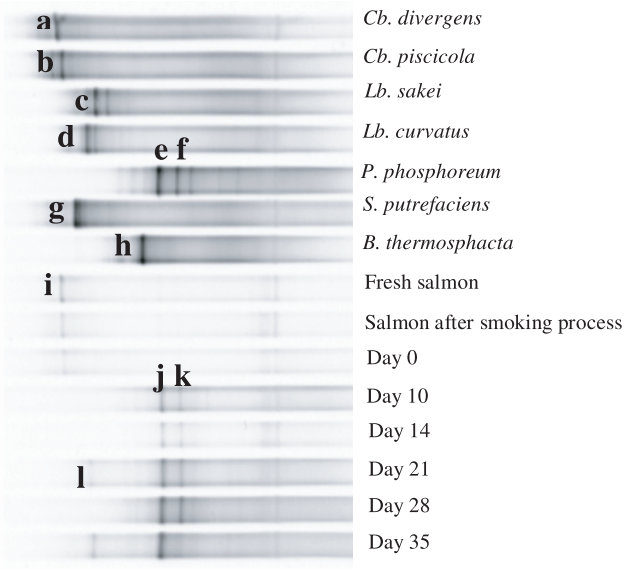

Figure 2. TTGE profile of Scottish CSS sample from smokehouse C.

as references: *Cb. divergens*, *Cb. piscicola*, *Lb. sakei*, *Lb. curvatus*, *B. thermosphacta*, *P. phosphoreum* and *S. putrefaciens*. These species showed different migration levels, allowing their differentiation. The TTGE profile of the Scottish CSS (C), sampled every week during storage, is shown in Figure 2. The migration level of V6-V8 DNA fragments amplified from CSS was compared with those of the seven bacterial species.

In fresh salmon, the fragment "i", showing the same migration level as fragment "a" corresponding to *Cb. piscicola,* was detected up to day 0 but disappeared at day 10. The appearance of 2 fragments, fragments "j" and "k", was observed at day 10. These fragments had the same profile and migration level as fragments "e" and "f", corresponding to *P. phosphoreum*. Another fragment detected was fragment "l", appearing at day 21. This showed the same migration level as fragment "d", corresponding to *Lb. curvatus*. In order to verify their identity, the fragments were extracted from the gel then cloned and sequenced. All sequences from each species matched with the sequences of corresponding species that are available in the GenBank database. These results validated the identification by migration level comparison.

4. DISCUSSION

Microbial ecology studies need fast and highly specific detection techniques. In this study, LAB from CSS were enumerated and identified by PCR-based techniques using species-specific primers or digestion using a restriction enzyme. The TTGE technique was evaluated in order to monitor the bacterial diversity in CSS.

The results showed that in fresh salmon, the Gram-positive bacteria population varied from 10^2 to 10^3 cfu·g^{-1} and decreased after the smoking process. The smoking process is followed by a maturation step for 1–4 days and by vacuum packaging. After the maturation step (day 0), Gram-positive bacteria reached 80 to 10^2 cfu·g^{-1} and increased during the 5-week storage, reaching 10^6–10^8 cfu·g^{-1}.

Carnobacterium, *Lactobacillus* and *Brochothrix* sp. are Gram-positive bacteria

found in CSS [12, 22]. *Carnobacterium* and *Lactobacillus* can be easily distinguished by their 16S-23S ISR profiles. Kabadjova et al. [10] have shown that *Carnobacterium* can be divided into 2 groups according to their ISR profiles, the *Cb. piscicola*/*Cb. gallinarum* group and the *Cb. divergens*/*Cb. mobile* group. Furthermore, these species can be distinguished by digestion using *Hin*f I, *Hin*d III or *Taq* I enzymes that generate different digestion profiles. In this study, the *Hin*d III enzyme was chosen, as previously reported [10]. *Cb. piscicola* and *Cb. divergens* were detected but they did not dominate the LAB population, except in the Scottish CSS. In contrast, *Cb. piscicola* has previously been identified as the dominant LAB species in CSS [6].

On the other hand, *Lactobacillus* can be easily identified by using species-specific primers designed by Berthier and Ehrlich [2]. The occurrence of *Lb. curvatus* as the main LAB (52–55%) has been reported in CSS, while *Lb. sakei*, *Lb. plantarum*, *Carnobacterium* spp. and *Leuconostoc* spp. were present in smaller numbers [22]. The presence of *Lb. sakei*, *Lb. farciminis* and *Lb. alimentarius* has also been detected in previous work [12]. In vacuum-packaged 'gravad' rainbow trout, *Lb. curvatus*, *Lb. sakei*, and *Cb. piscicola* were the major LAB species associated with spoilage of this product [14]. Characterisation of LAB from cold-smoked rainbow trout using ribotyping revealed that *Leuconostoc mesenteroides*, *Ln. citreum*, *Lb. sakei* and *Lb. curvatus* were the major species associated with spoilage of this fish product [13].

In this study, *Lb. curvatus* was found to be the dominant species at day 28 and day 35 in 3 of 5 samples (salmons A, B1 and B2). *Cb. piscicola*, *Cb. divergens* and *B. thermosphacta* were also identified during storage. These results are in agreement with previous work [22]. A significant variability between CSS samples was observed. Indeed, in the Scottish salmon (C), *B. thermosphacta* was the dominant species at day 35, while in the Norwich salmon (D), *Cb. piscicola* was mainly present. In contrast to previous works [12, 22], *Lb. sakei* was detected in only one CSS and no strains of *Lb. plantarum*, *Lb. farciminis* or *Lb. alimentarius* were identified in our samples. This fact may be due to the different CSS samples studied. The contamination of raw material and different technological process in smokehouses may affect the bacterial composition in CSS. Moreover, the identification results using traditional techniques might be different from those of molecular methods.

TTGE analysis was evaluated to reveal the bacterial diversity in CSS samples. The species frequently isolated in CSS such as *Cb. piscicola*, *Cb. divergens*, *Lb. sakei*, *Lb. curvatus*, *B. thermosphacta*, *P. phosphoreum* and *S. putrefaciens* were chosen as references [12, 22]. The comparison of migration level profiles and the sequences of different fragments generated from CSS samples and reference bacteria were used in order to identify the different bacteria species. The presence of a weak fragment corresponding to *Lb. curvatus* was observed at days 21 and 35 (Fig. 2). Meanwhile, *P. phosphoreum* was detectable from day 10 to day 35. The TTGE profiles indicated that LAB were less detectable than *P. phosphoreum* at the end of the storage. As previously described, PCR amplification on 16S rDNA may cause a bias, as only Gram-negative bacteria were revealed [3]. Various reasons could explain this fact. First, Gram-positive bacteria may be under-represented in the sample used [23]. This is not the case in our work as shown by the enumeration results (Tab. IV). Secondly, Gram-positive bacteria's DNA may be more difficult to extract. Thirdly, the universal primers may not be so universal since they are suitable when used on pure cultures, but are selective on mixed DNA. The fourth possible explanation is the presence of viable non-cultivable forms of certain bacterial species that are detectable by TTGE but not by culture methods. DNA extraction and PCR

bias are probably the major causes of the non-detection of species.

Finally, this study showed that LAB isolated from CSS during storage could be easily identified by specific primer-PCR or RFLP of 16S-23S rDNA ISR. In this work are presented the first results of TTGE used in order to monitor the modification of bacterial diversity in CSS during storage. All the bacteria species (*Cb. piscicola*, *Lb. curvatus* and *P. phosphoreum*) identified after culture could be specifically detected by TTGE. However, the detection of LAB seems to be under-evaluated as compared with the enumeration results. It would be interesting to complete this work by trying different DNA extraction methods, testing several primer pairs, and also by determining the sensitivity and detection limit of the TTGE technique.

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