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Characterization of the dominant bacterial communities during storage of Norway lobster and Norway lobster tails (*Nephrops norvegicus*) based on 16S rDNA analysis by PCR-DGGE

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ABSTRACT

The aim of this study was to investigate the microbial quality of whole Norway lobster (*Nephrops norvegicus*) and Norway lobster tails to optimize handling conditions. This was done by assessing the total viable count (TVC) and characterizing the dominant microbiota. The cultivable microorganisms were quantified via classical microbiological plating methods. To characterize as many bacterial species present as possible, we performed advanced molecular identification techniques (PCR-DGGE). The initial TVC of fresh Norway lobster meat was high (3.0 log cfu/g) as compared to fish. No significant difference between whole Norway lobster and Norway lobster tails could be found during the storage period. From day 6 of storage, a significant difference between Plate Count Agar (PCA) and Marine Agar (MA) was observed. The microbiota of Norway lobster was dominated by members of the Gram-negative genera such as *Psychrobacter* spp., *Pseudoalteromonas* spp., *Pseudomonas* spp., *Luteimonas* spp., and *Aliivibrio* spp. From these bacteria, mainly *Psychrobacter* spp. and *Pseudomonas* spp. remained present until the end of the storage period. These are known spoilage organisms in fishery products. Other known spoilage organisms of crustaceans such as *Photobacterium* spp. could not be identified.

1. Introduction

Fish meat is highly prone to spoilage caused by microorganisms. In contrast to white fish, crustaceans possess more free amino acids (a readily available bacterial growth substrate), which makes them even more susceptible to spoilage. Spoilage causes amines, sulfides, alcohols, aldehydes, ketones and organic acids to be produced, causing undesirable off-odors and off-flavors. In general, the initial microflora of fishery products is dominated by Gram-negative, rod-shaped bacteria of the genera *Photobacterium*, *Vibrio*, *Shewanella*, *Pseudomonas*, *Pseudoalteromonas*, *Moraxella*, *Acinetobacter* and *Flavobacterium* (Gram and Huss, 1996). However, only a small number of these microorganisms participate in the spoilage process. This group of organisms is often referred to as the specific spoilage organisms (SSOs) (Dalgaard, 1995; Gram and Dalgaard, 2002). Many fish species contain trimethylamine oxide (TMAO) as part of the non-protein-nitrogen fraction. SSOs such as *Aeromonas* spp., *Photobacterium phosphoreum*, *Shewanella putrefaciens*-like organisms, Enterobacteriaceae and *Vibrio* spp. are all capable of reducing TMAO to trimethylamine (TMA), causing the typical off-odor of spoiled fish. H_2S-producing bacteria are also found to be important spoilage organisms of chilled and aerobically stored fresh fish (Gram et al., 1987). Various bacteria such as *S. putrefaciens*, types of Enterobacteriaceae, Aeromonaceae, Vibrionaceae and some *Lactobacilli* are able to produce H_2S. In contrast, neither *Pseudomonas* spp. nor *P. phosphoreum* produce significant amounts of H_2S (Gram and Huss, 1996; Lyhs, 2009). The growth of different SSOs depends on several parameters: food product, type of preservation, temperature, atmosphere, and salt content, among others. Typical spoilage bacteria for fresh chilled fish stored in air are *Pseudomonas* spp. and *S. putrefaciens*.

Norway lobsters (*N. norvegicus*) are landed in the Belgian harbors as either whole products or as Norway lobster tails. Depending on the damage during fishing, Norway lobster tails are sometimes removed after catch and the heads discarded. To our knowledge, no information is available on the influence of this tailing procedure on the composition of the bacterial population and on the shelf-life of the product.

Characterization of spoilage organisms and SSOs is often done via classical microbial plating techniques. These cultivation-

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dependent methods have limitations, however: bacteria in general, and particularly those of marine origin, are not able to grow on all media (Broekaert et al., 2011). This selectivity might hamper the full characterization of the spoilage organisms, which may lead to inadequate measures being taken to prolong shelf life. Only a minor selection of microorganisms can be cultivated on marine environmental samples (Joint et al., 2010) but food is a rich substrate. We can therefore suppose that most of the microorganisms are cultivable on an appropriate substrate (Cambon-Bonavita et al., 2001; Gram and Dalgaard, 2002). Advanced molecular methods now allow identification of non-cultivable bacteria. Using molecular methodology at the end of shelf life of sea bream, Parlapani et al. (2013) were able to find *Aeromonas* sp. as part of the dominant microbiota. That species had not been previously detected using classical methods. By combining advanced molecular techniques with the classical plating methods for detecting potential SSOs, one can obtain a more accurate picture of the microbiota present on seafood samples.

PCR-DGGE is a suitable technique for studying the bacterial composition of different habitats, soils, foods and water samples. Earlier PCR-DGGE studies on fish were performed on Atlantic mackerel (Svanevik and Lunestad, 2011), Atlantic salmon, cod, farmed halibut (Hovda et al., 2007a,b,c, 2012) and tuna (Chongtao et al., 2012), among others. To the best of our knowledge, the present study is the first to determine the diversity of initial and spoilage microbiota of Norway lobster using DNA extraction directly from flesh.

The aim of this study was to investigate the influence of tailing on the microbial quality of the tailed lobster product and to characterize the dominant microbiota on both whole and tailed lobsters by applying the combination of conventional microbiological methods and culture independent molecular methods. To increase the relevance of the findings, the Norway lobster samples were collected during a commercial catch situation. Understanding the spoilage of Norway lobster can facilitate the development of detection methods and improve preservation techniques that target the SSOs present. We expect this to result in better quality and longer shelf life.

2. Materials and methods

2.1. Norway lobster treatment

Norway lobsters were caught in March 2013 in the North Sea (56°16′5″N, 05°30′0″E) by a commercial beam trawler (N350). Trawl duration lasted for 6 h. Two kg of living Norway lobsters were kept in ice and transported to the laboratory. Most lobsters were still alive upon arrival at the laboratory, where they were divided randomly into two subsamples. From one subsample, tails were removed from the raw Norway lobsters by twisting the abdomen from the cephalothorax according to common practice aboard fishing vessels. The whole lobsters died during ice storage. The samples were then kept within melting ice in a refrigerator at 2 ± 1 °C for two weeks. Ice was replaced as necessary. On days 1, 3, 6, 8, 10, 13 and 15 of storage, the total viable count (TVC) was determined on Plate Count Agar (PCA) and Marine Agar (MA). For each sampling point, three Norway lobsters were analyzed per treatment. Characterization of the microbial population was performed on bacterial DNA from the PCA and MA plates (bulk cell DNA samples) and via direct DNA sampling on days 1, 6 and 15 of storage.

2.2. Total viable count on MA and PCA

The tail meat of the lobsters was removed aseptically and transferred to a Stomacher® bag containing the appropriate volume (1/9) (w/v) of sterile Maximum Recovery Diluent (MRD) (Oxoid, UK). The mixture was homogenized twice during 120 s with a microbiological homogenizer (BagMixer, Interscience, France). A series of tenfold dilutions were made (up to 10^{-5}) in MRD and aliquots of 0.1 ml of the dilutions were spread in duplicate on dry Plate Count Agar (Oxoid, CM0325B) and Marine Agar (Difco, MA 2216) plates. PCA was chosen as general reference medium for the enumeration of microbiota on all food and feed (ISO 17410, 2001). MA was used as this medium gave the best quantitative (TVC) and qualitative results (replication and DGGE analysis) in the study of Broekaert et al. (2011). With a few exceptions, most microorganisms were capable of growing on this medium. The plates were incubated for 72 h at 22 °C and colony forming units (cfu) were counted. Results were normalized by conversion into logarithmic values ($\log_{10}$ cfu/g). Student's *t*-test was used to determine whether the results of total viable count on the media were significantly different.

2.3. Collection of bacteria from MA and PCA plates (bulk cell DNA)

After counting, a complete plate swab using an inoculation loop was taken from Marine Agar (MA) and PCA plates at days 1, 6 and 15 and placed in an Eppendorf tube. The pellets were washed twice with 1× Phosphate Buffered Saline (137 mM NaCl, 2.7 mM KCl, 0.9 mM KH_2PO_4, 6.4 mM Na_2HPO_4 (pH 7.4)) and frozen at −20 °C. The pellets were thawed immediately prior to DNA purification.

2.4. Extraction of bacterial DNA directly from lobster material (direct DNA)

Fifty milliliters of the homogenized mixture in MRD (used to count the total bacteria; see Section 2.2) was collected and stored at −20 °C on days 1, 6 and 15. The extraction of DNA directly from lobster was done according to the procedure of Rudi et al. (2005). Briefly, the homogenates were thawed, centrifuged to pellets and supernatant was recovered to purify the bacterial DNA.

2.5. DNA purification

DNA from the plates and from the lobster material was purified using the commercially available DNeasy® Blood and Tissue Kit (Qiagen, Germany, Cat. No. 69506) according to the manufacturer's instructions. Samples were pretreated with a lysozyme buffer to ensure the extraction of Gram-positive bacterial DNA. The purity of the samples was measured using the Nanodrop TM 1000 spectrophotometer (Thermo Scientific).

2.6. PCR protocol

One microliter of the DNA sample was used as template for the PCR. The forward primer 5′ CC TAC GGG AGG CAG CAG 3′ including a 40 base GC-clamp at the 5′ end. The reverse primer 5′ ATT ACC GCG GCT GCT GG 3′ was used to amplify the hypervariable V3 region of the 16S rDNA (Muyzer et al., 1993). The forward and reverse primers were used to amplify the 16S rDNA regions in the various bacterial species that respond to positions 341–534 in *Escherichia coli*. The V3 region is a good choice in terms of length and species–species heterogeneity. The amplification resulted in DNA fragments of approximately 240 bp. The Faststart® Taq DNA polymerase kit (Roche, 12 032 953 001) was used for the PCR. The PCR mix (50 μl per reaction) was composed of 5 μl of 10× PCR buffer, 1 μl of dNTPs (200 μM final concentration), 0.4 μl Taq polymerase (2 U/50 μl reaction), 10 μl of GC-rich solution, 1 μl (0.2 mM final concentration) of each primer, and 30.6 μl of ddH_2O. The reactions

were performed on a Thermocycler (Biometra, Germany). A touchdown PCR was performed according to Muyzer et al. (1993) with slight modifications as follows. During the first cycle an annealing temperature of 63 °C was processed instead of 65 °C and the additional 12 cycles were carried out at 55 °C. A final extension of 7 min at 72 °C was performed. The PCR product length was verified on a 1% agarose gel (LE, analytical grade, Promega), stained with Gelred™ nucleic acid stain (Biotium, USA), and visualized and photographed under UV light (UVtransilluminator 265 nm TFX20M).

2.7. DGGE analysis

Before DGGE analysis, the PCR products were purified using ethanol precipitation (Sambrook and Russell, 2001). DGGE was performed with the INGENY phor-U-2 unit using 10 μl of the purified and concentrated PCR product on a 0.75 mm thick 8% (w/v) polyacrylamide gel (acrylamide–bisacrylamide, 37.5:1) in 1× TAE buffer which contained a 45–65% urea-formamide denaturing gradient (100% corresponding to 7 M urea and 40% (w/v) formamide). Electrophoresis was run in 1× TAE buffer at 60 °C for 16 h at 120 V. After electrophoresis, the gel was stained with 1× Sybr® Gold (Invitrogen, USA) in 1× TAE during 30 min before visualization of the fragments under UV illumination as described above. The DGGE marker I (5 fragments) NIPPON gene (Wako company, Japan) was used as reference.

2.8. Sequencing of the DGGE fragments and identification of the bands

DGGE-separated DNA fragments to be sequenced were punctured with sterile pipette tips and transferred into 10 μl sterile water. The DNA diffused into the water during a few hours at room temperature. Two microliters were then used as a template and re-amplified by PCR as described above with the same primers omitted from their GC clamp. PCR products were cleaned with the MSB® Spin PCRapace kit (Invitek, Germany) following the manufacturer's protocol. Sequencing was performed at Macrogen (Amsterdam, the Netherlands). Sequencing reactions were performed in the DNA Engine Tetrad 2 Peltier Thermal Cycler (Bio-Rad, USA) using the ABI BigDye Sequencing Kit (Applied Biosystems, USA), following the protocols supplied by the manufacturer. Single-pass sequencing was performed on each template. Both forward and reverse fragments were sequenced using the same primers as for PCR. Searches in BLAST from Genbank/NCBI were performed to identify the bacterial species. Sequence match with 97% similarity or above was considered to represent the same species according to the species identification protocol (Gevers et al., 2005).

3. Results and discussion

Norway lobsters and Norway lobster tails were collected from a beam trawl fishing boat to characterize the bacterial biota and to describe the changes in bacterial load during storage of the products. Characterization was performed using conventional microbiological quantification as well as PCR-DGGE. Bacterial DNA was extracted either directly from the lobster flesh without a prior cultivation step or from bacterial cells after cultivation on MA and PCA.

3.1. Total viable counts

TVC was performed on PCA and MA for lobster tails and whole lobster (Fig. 1). No significant differences ($p > 0.05$) between whole Norway lobster and Norway lobster tails were found for the total plate count on PCA or MA. The results are therefore presented together. The effect of tailing (which removes the internal bacteria of the stomach) did not result in any reduction on the formation of bacteria. It is known, however, that removal of the internal organs of fishery products can result in longer shelf life for some species. A prolongation of the shelf life was noticed for haddock, saithe, plaice (Karl and Meyer, 2007) and aquacultured sea bass (Papadopoulos et al., 2003; Paleologos et al., 2004). For other species, no effect or a negative effect was observed. Gutting European hake had a detrimental effect on its shelf life in ice (Baixas-Nogueras et al., 2009). When removing the internal bacterial content by gutting, an increase of the scattering of the microbial load in the muscles can take place. In our study, the stomach was removed by tailing, but not the intestinal tract. This can explain why no influence was seen on the bacterial load in the flesh as the inner bacterial content of the intestines was still present. Furthermore, tailing exposes the muscular masses to external conditions and can thus favor entrance of microorganisms. In this way, removing the stomach bacteria can

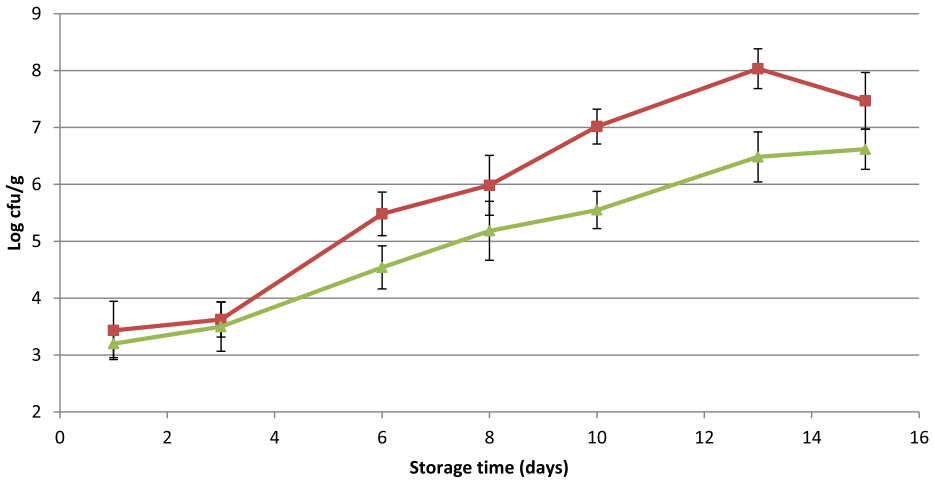

Fig. 1. Growth of bacteria in Norway lobsters (whole and tails) on MA and PCA. Samples were stored for a total of 15 days in ice (2 ± 1 °C). ■ Norway lobsters on MA, ▲ Norway lobsters on PCA.

be compensated by entrance of external bacteria. From day 6 of storage, a significant difference ($p < 0.05$) in total aerobic plate count between PCA and MA was noted. These results confirm earlier publications, indicating differences up to one log or more between PCA and other growth media such as Iron Agar (IA), Long and Hammer agar (LH) or Marine Agar (MA) for marine fish species (Lyhs, 2009; Broekaert et al., 2011; Van Spreekens, 1974). This underscores the importance of selecting the appropriate growth media. However, this difference is only noticeable after 4 days of storage. The initial bacterial load of fresh Norway lobster flesh was high (3.4 ± 0.5 log cfu/g on MA and 3.2 ± 0.2 on PCA) as compared to fish, which can show a very low bacterial load (10 cfu/g) at the time of death (Huss, 1995; Nuin et al., 2008). These results are in agreement with those obtained in other studies on Norway lobster (Gornik et al., 2011; Albalat et al., 2011; Boziaris et al., 2011). Crustaceans rely on the innate immune system (Terwilliger, 2007) which is less efficient than the acquired immune system. Therefore it is possible that bacteria invading body cavities of live lobster are removed less efficiently than in vertebrates (Gornik et al., 2011). This may explain the high initial bacterial load of Norway lobster as compared to fish. The initial concentration remained stable for three days, then increased steadily to reach 7.5 ± 0.5 log cfu and 6.6 ± 0.3 log cfu at day 13 on MA and PCA, respectively. On day 15 of storage, the increase in number of bacteria slowed down, indicating that bacterial cells had reached their stationary phase. Freshness assessment of lobster is often performed using sensory testing. Rejection of ice-stored Norway lobster in sensory tests was found to occur at day 7 by Albalat et al. (2011). In our study, rejection at day 7 corresponds to the end of shelf life at a total viable count of 6 log cfu/g on MA and 5 log cfu/g on PCA. These values are lower than the food quality total viable count norm of 7 log cfu/g for the assessment of the end of shelf life of raw seafood products (ICMSF, 1986).

3.2. 16S rDNA based microbial identification

The bacterial profile of the stored samples changed during the storage period (Fig. 2). On the first day of storage, the DGGE profile is characterized by numerous bands (*e.g.* 10 for bulk cell DNA from MA), which disappear during storage. Fewer bands can be seen when the direct DNA method was used due to a lower concentration of bacterial DNA at the beginning of the storage period. Table 1 shows all the identified bands. Only ten taxa were found. Table 2 shows in which samples these taxa were present. *Pseudoalteromonas* spp. were present in the beginning of storage, in addition to *Pseudomonas* spp. and *Shewanella* spp. on Norway lobster tails, and *Psychrobacter* spp. and *Vibrio harveyi* on whole Norway lobster as shown from 16S rDNA sequence analysis. On day 6, the bacterial diversity on whole Norway lobster was more complex than on tails. On tails, the numerous bands corresponding to a high variety had diminished and only *Psychrobacter* spp. were still present. On whole Norway lobster the same bacteria were found in addition to *Pseudomonas* sp., *Allivibrio* sp. and *Pseudoalteromonas* sp. At the end of the storage period, *Psychrobacter* sp. and *Pseudomonas* sp. on both whole Norway lobster and tails dominated the bacterial flora. *Luteimonas* sp. was present only on tails and *Pseudoalteromonas* sp. and *Microbacterium arborescens* only on whole Norway lobster.

Mainly *Pseudomonas* spp. and *Psychrobacter* spp. were detected in most of the samples at the end of storage. This suggests that one or both of these bacteria contribute considerably to the overall spoilage of aerobically stored Norway lobster. *Pseudomonas* spp. are well-known spoilage bacteria of ice stored marine and freshwater fish (Gram, 1992; Gram and Huss, 1996). Their importance in spoilage of fish species from the Mediterranean Sea has been reported and their growth strongly correlates with the decrease of sensory quality (Tryfinopoulou et al., 2002; Koutsoumanis et al., 2002; Taoukis et al., 1999; Koutsoumanis and Nychas, 1999).

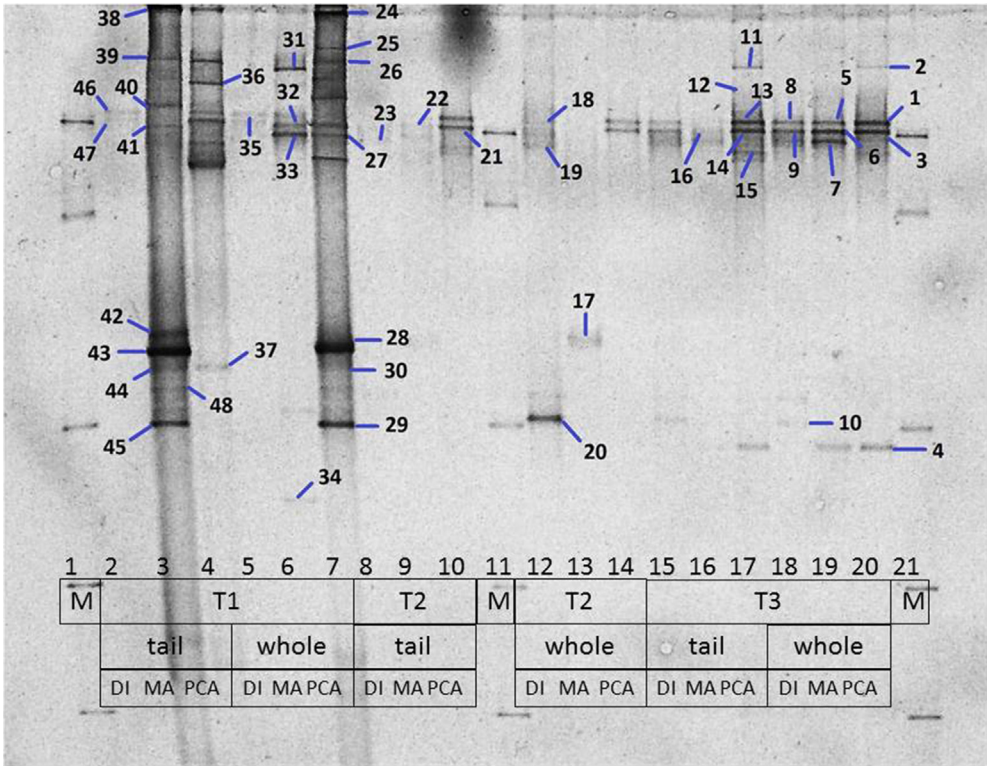

Fig. 2. DGGE-based bacterial profile of whole Norway lobster (whole) and Norway lobster tails (tail) during storage for 15 days (T1 = day 1 of storage, T2 = day 6 of storage, T3 = day 15 of storage) from direct DNA extraction (DI), bulk cell DNA from MA (MA) and bulk cell DNA from PCA (PCA). M corresponds to the internal marker.

Table 1
16S rDNA sequence similarities to closest relatives of DNA recovered from the respective bands in the DGGE gel (Fig. 2).

Band no.	Closest relative in BLAST	Similarity (%)	Accession no.
1	*Pseudomonas* sp.	99%	KC687078.1
2	*Psychrobacter* sp.	100%	KC753336.1
3	*Pseudomonas* sp.	92%	JN975150.1
4	*Microbacterium* sp.	97%	KC434984.1
5	*Citrobacter* sp.	96%	KC879274.1
6	*Psychrobacter* sp.	98%	800050.1
7	*Pseudoalteromonas* sp.	98%	FJ386507.1
8	*Pseudomonas* sp.	100%	KC687078.1
9	*Psychrobacter* sp.	100%	KC753336.1
10	*Pseudomonas* sp.	91%	AB812783.1
11	*Pseudoalteromonadaceae* sp.	93%	AB375517.1
12	*Psychrobacter* sp.	93%	EU781522.1
13	*Pseudomonas* sp.	100%	KC687078.1
14	*Pseudomonas* sp.	100%	GU191929.1
15	*Luteimonas* sp.	99%	JQ349045.1
16	*Psychrobacter* sp.	96%	EU375114.1
17	*Pseudoalteromonas* sp.	100%	AB819713.1
18	*Aliivibrio* sp.	97%	AB550537.1
19	*Aliivibrio* sp.	99%	AB550537.1
20	*Pseudoalteromonas* sp.	100%	AB819713.1
21	*Pseudomonas* sp.	95%	AF321025.1
22	*Psychrobacter* sp.	97%	KC753350
23	*Aeromonas* sp.	93%	JQ912607.1
24	*Pseudoalteromonas* sp.	98%	EU770397
25	*Pseudoalteromonas* sp.	98%	AB819713.1
26	*Pseudoalteromonas* sp.	98%	AB819713.1
27	*Azotobacter* sp.	81%	JN692282.1
28	*Pseudoalteromonas* sp.	99%	KC899232.1
29	*Pseudoalteromonas* sp.	98%	EU770397
30	*Pseudoalteromonas* sp.	99%	AB819713.1
31	*Psychrobacter* sp.	100%	C753336.1
32	*Vibrio* sp.	100%	EU834009.1
33	*Psychrobacter* sp.	99%	KC753336.1
34	*Pseudoalteromonas* sp.	100%	EU433337.1
35	*Pseudoalteromonas* sp.	98%	AB819713.1
36	*Shewanella* sp.	97%	JQ861989.1
37	*Pseudoalteromonas* sp.	97%	DQ873753.1
38	*Pseudomonas* sp.	100%	KC898257.1
39	*Pseudoalteromonas* sp.	99%	AB819713.1
40	*Pseudoalteromonas* sp.	99%	AB819713.1
41	*Pseudoalteromonas* sp.	100%	FJ984925.1
42	*Pseudoalteromonas* sp.	99%	B819713.1
43	*Pseudoalteromonas* sp.	100%	AB819713.1
44	*Pseudoalteromonas* sp.	98%	AB819713.1
45	*Pseudoalteromonas* sp.	100%	AB819713.1
46	*Pseudoalteromonas* sp.	99%	KC899232.1
47	*Pseudoalteromonas* sp.	96%	AB819713
48	*Pseudoalteromonas* sp.	100%	AB819713.1

These microorganisms were also reported responsible for quality changes and development of sweet, fruity off-odors in chilled fish from the North Atlantic Ocean (Olafsdottir et al., 2006; Reynisson et al., 2009; Lauzon, 2000).

Species of the genus *Psychrobacter* are well-known spoilers of chilled protein-rich foods. In seafood, they are less associated with seafood spoilage as they seem to be unable to compete with common spoilage organisms (Rodriguez-Calleja et al., 2005; Gennari et al., 1999). They have been isolated on several seafood products such as fresh peeled and unpeeled brown shrimp, ray (Broekaert, 2012), tropical and Nordic cooked and peeled shrimp (Mejholm et al., 2005; Jaffres et al., 2009), salt-cured cod (Bjørkevoll et al., 2003) and shellfish (Prapaiwong et al., 2009). Study of the spoilage potential of *Psychrobacter immobilis* showed that these are able to produce only weak fishy and musty off-flavors (Mejlholm et al., 2005; Prapaiwong et al., 2009). *Psychrobacter cibarius* shows little spoilage potential on peeled and unpeeled brown shrimp (Broekaert et al., 2013). In contrast, the work of Bjørkevoll et al. (2003) suggests that *Psychrobacter* sp. is responsible for the musty odor in rehydrated salt-cured and dried salt-cured cod, resulting in a sensorial unacceptable product. The high salt content in the product favored the maximum growth rate of *Psychrobacter*. A study on ice-stored ray (Broekaert, 2012) indicates a co-dominance of *Pseudomonas* spp. with *Psychrobacter cryohalolentis* and *P. cibarius*, which are capable of producing Volatile Organic Compounds associated with spoilage. They were also capable of breaking down urea. Further research into the quantitative ability to produce spoilage metabolites from *Psychrobacter* spp. and *Pseudomonas* spp. on Norway lobster should be performed in order to confirm their participation in the spoilage process of Norway lobster.

The community of spoilage bacteria in our study agreed with the work of Boziaris et al. (2011), who found (using classical plating methods) that *Pseudomonas* sp. was the main spoilage organism of air-stored Norway lobster from Greek temperate waters. On the other hand, Gornik et al. (2011) identified *P. phosphoreum* as the main spoilage organism in ice-stored Norway lobster of the Clyde Sea area. It is well known that the initial bacterial composition can change with geographical origin, thus inducing different spoilage patterns (Gram and Huss, 1996). The bacterial community can also vary with sampling time as related to temporal variations in food supply.

Due to co-migration of the DGGE bands, not all bands in the profiles have been sequenced and analyzed. This may result in some biologically significant bacteria remaining undiscovered. This issue of the DGGE technique has been described in different studies (Jackson et al., 2000; Hovda et al., 2007a; Li et al., 2006). Nevertheless, sequencing of individual colonies (unpublished results) on MA and PCA plates never revealed the presence of *Photobacterium* sp. Limitations of the DGGE method include the detection limit, which varies between 10^3 cfu/g and 10^8 cfu/g depending on the species and on the other members of the microbial community (Ercolini, 2004). This can possibly explain the low yield of bands in DNA samples directly extracted from Norway lobster meat, especially in the beginning of the storage period. This indicates that using molecular based methods to the exclusion of cultivation can lead to erroneous characterization of the bacterial biota.

Furthermore, the observed bands in one lane of the profile do not necessarily correspond to different bacteria. It is well established that some bacteria have heterogeneous copies of rDNA operons, which may cause the diversity to be overestimated (Nübel et al., 1996; de Araujo and Schneider, 2008). In this study, *Pseudoalteromonas* sp. was represented by numerous bands. They cannot be assigned as different species with certitude unless further identification to species level is performed. Using DGGE, identification to species level is limited, however, because the fragments to be resolved cannot be longer than 500 bp (Ercolini, 2004). Some analyzed bands had sequence similarity of less than 97% and were thereby not assigned as the same species.

4. Conclusion

Based on the Total Viable Count (MA and PCA) assay, no significant difference was observed between the storage of whole Norway lobster or lobster tails. The microbiota of Norway lobster was dominated by members of the Gram-negative genera as *Psychrobacter* spp., *Pseudoalteromonas* spp., *Pseudomonas* spp., *Luteimonas* spp., *Aliivibrio* spp., *Microbacterium* spp., *Vibrio* spp., *Shewanella* spp. Examination by PCR-DGGE of the bulk cell sample after cultivation gave a higher number of taxa as compared to samples without prior cultivation, especially at the beginning of the storage period. At the end of the storage period, less variety in microbiota and a shift toward *Psychrobacter* sp. and *Pseudomonas* sp. was observed. *Pseudomonas* sp. was detected as part of the

Table 2
Results after sequencing of the dominant bands from the bacterial profiles of Fig. 2 in the different storage variants and during the storage period. Numbers correspond to the bands in Table 1 and Fig. 2.

Storage days	Norway lobster tails			Whole Norway lobster		
	Direct DNA from lobster	Bulk cell DNA from MA	Bulk cell DNA from PCA	Direct DNA from lobster	Bulk cell DNA from MA	Bulk cell DNA from PCA
0	*Pseudoalteromonas* sp. (#46)	*Pseudoalteromonas* sp. (#39–45, 48) *Pseudomonas* sp. (#38)	*Pseudoalteromonas* sp. (#37) *Shewanella* sp. (#36)	*Pseudoalteromonas* sp. (#35)	*Pseudoalteromonas* sp. (#34) *Psychrobacter* sp. (#31, 33) *Vibrio* sp. (#32)	*Pseudoalteromonas* sp. (#30, 29, 28, 26, 25, 24)
6		*Psychrobacter* sp. (#22)		*Aliivibrio* sp. (#18,19) *Pseudoalteromonas* sp. (#20)	*Pseudoalteromonas* sp. (#17)	*Pseudomonas* sp. (#13) *Psychrobacter* sp. (#6)
15	*Pseudomonas* sp. (#8) *Psychrobacter* sp. (#9)		*Pseudomonas* sp. (#13) *Pseudomonas* sp. (#14) *Luteimonas* sp. (#15)	*Pseudomonas* sp. (#8) *Psychrobacter* sp. (#9)	*Pseudoalteromonas* sp. (#7) *Psychrobacter* sp. (#6)	*Pseudomonas* sp. (#1) *Psychrobacter* sp. (#2) *Microbacterium* sp. (#4)

The associated bands from Fig. 2 and Table 1 are shown in brackets. Only sequences with similarity >97% are included in the table.

observed bacterial community at the end of storage as well as *Psychrobacter* sp. These are known spoilage organisms in fishery products. Another common spoilage organism of crustaceans, *Photobacterium* sp., was not observed in this study. Future research into the spoilage potential of the organisms found should be conducted to confirm their participation in the decay process of Norway lobster.

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