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American lobsters (*Homarus americanus*) not surviving during air transport: evaluation of microbial spoilage

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Abstract

Eighteen American lobsters (*Homarus americanus*), dead during air transport, were analysed in order to evaluate the microbial population of meat, gills and gut: no specific studies have ever been conducted so far on the microbiological quality of American lobsters' meats in terms of spoilage microbiota. The meat samples showed very limited total viable counts, in almost all the cases below the level of 6 Log CFU/g, while higher loads were found, as expected, in gut and gills, the most probable source of contamination. These data could justify the possibility to commercialise these not-surviving subjects, without quality concerns for the consumers. Most of the isolates resulted to be clustered with type strains of *Pseudoalteromonas* spp. (43.1%) and *Photobacterium* spp. (24.1%), and in particular to species related to the natural marine environment. The distribution of the genera showed a marked inhomogeneity among the samples. The majority of the isolates identified resulted to possess proteolytic (69.3%) and lipolytic ability (75.5%), suggesting their potential spoilage ability. The maintanance of good hygienical practices, especially during the production of ready-to-eat lobsters-based products, and a proper storage could limit the possible replication of these microorganisms.

Introduction

The 90% of fish international market is based on processed products, but live crustaceans, especially clawed and spiny lobsters, crabs, marine and freshwater prawns and freshwater crayfish, are really appreciated for their high nutritional and commercial importance (Fotedar and Evans, 2011). The American lobster, *Homarus americanus*, is one of the most important commercial species marketed internationally and one of the major economic resource of the coastal communities of North America, with more than 130,000 tons of alive animals commercialised worldwide in 2006 and producing nearly 545 million of euros of profits. Due to the decrease of the European lobster *Homarus gammarus* catching figures, American lobsters are imported by several European countries, especially Mediterranean ones: in Italy about 4387 tons are annually introduced for an economic value of 44 million of euros (Barrento *et al.*, 2009; FAO, 2006). American lobsters are mainly commercialised alive and cooked before consumption. After the capture and during the air transport, they are submitted to several stresses like air exposure, changes of the natural environments in terms of physical and chemical parameters such as water salinity and temperature, hypoxia, fasting, interactions with other conspecifics and human handling. These stressors can affect their health, reducing their quality, and in some cases could be lethal. Opposite opinions are still existing about the edibility of lobsters dead during air shipping: some sellers reject to sale the dead subjects, some others use these ones for cooked products, others sell these animals depreciated. At the moment there are no available studies about the hygienic quality of the living and dead animals, in terms of presence of potential pathogenic bacteria and of microbial populations. However, we could suppose that the loads and the typology of microbiota of lobsters reflect a combination of factors including the environment where they are captured, their feeding and living practices, the season of capture as well as the quality and temperature of the waters where they lived, as already demonstrated for other crustaceans and for finfish (Liston, 1980; Shewan, 1977). After death, once the immune response fails to function and the muscular structure declines due to microbial activity, the alteration begins quickly: generally this situation does not expose consumer to microbiological risks as these animals are mainly consumed after cooking. However, the increasing demand for ready-to-eat raw or cooked crustaceans and of fresh lobster tails could enhance the risk posed by these products, if subjected to improper storage or poor hygienic practices during production and commercialisation. Swartzentruber *et al*. (1980) found high total bacterial count values [more than 5 Log colony forming unit (CFU)/g] in 51.9% of frozen lobster tails samples, even if very low levels of coliforms and *E. coli* were detected. Although, as already mentioned, no studies till now were conducted on lobster meat bacterial population, generally the microbiota of crustaceans captured in temperate waters includes *Pseudomonas* spp., *Shewanella putrefaciens* and members of the group *Acinetobacter-Psychrobacter* (Gram and Huss, 1996).

Aim of our study was the microbial evaluation of the meat of American lobsters not surviving the air shipping from the harvest (USA) to a seafood distribution platform of an international wholesaler, located in Northern Italy, and the identification of the most representative bacterial populations in order to establish the major genera/species involved with microbial spoilage of these products. We also evaluated the microbial loads of gut and gills as possible sources of contamination.

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Key words: Dead American lobsters; Shipping; Food spoilage; Microbial population.

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Materials and Methods

Microbiological analyses

For this study, eighteen lobsters arrived dead after air transport lasted about 24 h from harvest (USA) to the seafood distribution platform, were collected. The subjects were transported at refrigeration temperatures in cardboard boxes containing ice gel packs where they were maintained separately thanks to

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cardboard dividers. The dead subjects were transported under refrigerated conditions to the laboratory where they were analysed immediately. Aseptically, gills, meat and intestine were withdrawn and submitted to microbiological analyses: 5-10 g of each sample were diluted 1:10 in saline diluent (NaCl 10 g/L, $MgSO_4 \cdot 7H_2O$ 1.53 g/L, $MgCl_2 \cdot 6H_2O$ 1.28 g/L, KCl 0.19 g/L, tryptone 1 g/L) and serial 10-fold dilutions were prepared. Total viable count (TVC) was enumerated onto modified Tryptose agar as described by Bernardi *et al*. (2015), incubated at 20°C for 72 h. The number of *Enterobacteriaceae* was determined according with ISO 21528-2:2004 method (ISO, 2004). Colonies of fluorescent pseudomonads were observed on plates of King's B agar (King *et al*., 1954) in ultraviolet light, after growth for 1-3 days at 20°C. Bioluminescent bacteria were enumerated onto glycerol based marine agar (GMA) (Makemson *et al.,* 1992); plates were incubated at 20°C for 72 h and observed daily in dark. Finally H_2S producing microorganisms were enumerated onto Iron Agar containing sodium thiosulphate (IA1 following Gram *et al.,* 1987) using the pour plate technique. Plates were incubated at 20°C for 72 h and observed for black colonies.

Phenotypical tests

From the TVC plates of meat samples, a number of five colonies for each of 12 subjects (identified with code from A to N) were randomly picked and subcultured onto modified Tryptose agar. The total of 60 isolates were afterwards submitted to the tests as described below.

The following reactions and biochemical tests were used for the characterisation of the isolates. Gram-reaction was tested by the KOH method (Gregersen, 1978). Phase contrast microscopy was used for the determination of shape and motility. Cytochrome-oxidase was tested with the method described by Kovacs (1956) and catalase formation with 3% H_2O_2.

The carbohydrate metabolism was observed in tubes of Hugh and Leifson medium (Hugh and Leifson, 1953). Tubes were stab inoculated (after boiling to avoid oxygen) and incubated at 20°C for a week. Fermentative bacteria produced acid from glucose, changing the color of the indicator from green to yellow, in all the volume of the medium. Oxidative bacteria grew only on the surface of the medium, and the eventual change of color (blue due to alcalinisation or yellow for acidification) was located within 2-3 cm from the surface.

Proteolytic activity was detected by gelatin hydrolysis on Frazier's agar plates (Frazier, 1926). The inoculated plates were incubated at 20°C for a week, than flooded with Frazier's reactive. Gelatinase activity was indicated by formation of a clear zone around the positive colonies.

Lipolytic activity was assessed on Sierra's agar containing Tween 80 (Sierra, 1957). The plates were incubated at 20°C for a week; positive strains showed a visible precipitation around the colonies.

All the isolates were screened for their ability to produce H_2S. The medium used was Iron agar 1 (Gram *et al*., 1987); each plate was prepared with 40 mL of agar stab inoculated. H_2S production was indicated by the formation of a black precipitate of FeS. To enable the growth of halophilic microorganisms, the original composition of some media (Iron Agar 1) were added of the following final salt concentrations: NaCl 10 g/L, $MgSO_4 \cdot 7H_2O$ 1.53 g/L, $MgCl_2 \cdot 6H_2O$ 1.28 g/L, KCl 0.19 g/L.

The ability to growth at 5, 30, 37 and 42°C was tested isolating each of the strain onto plates of modified Tryptose agar. Moreover the ability to grow onto modified tryptose agar including different salt concentration (0 and 0.5%) was evaluated preparing the plates with the different percentages of salt included.

Sequencing of 16s *rRNA* gene

Isolates were then identified by 16s *rRNA* gene sequencing (Eurofins, Berlin, Germany) using the standard forward primers CC-CD as described by Rudi *et al*. (1997). The sequences were analysed and compared to BIBI and NCBI-Blast (http://blast.ncbi.nlm.nih.gov/Blast.cgi) databases and the closest type strains relatives based on partial 16S *rRNA* gene sequences were determined. In order to verify if the number of identified taxa was representative of the whole microbial species present in the ecological niche of American lobsters meat, the total number of bacterial species and the percentage detected were estimated by method described by Chao *et al*. (2009) for incidence data.

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Results and Discussion

A total of 18 lobsters dead during shipping were analysed: meat, gills (as the main access point for bacteria) and gut bacterial contaminations were screened. The Food and Drug Administration (FDA, 2013), which has issued guidelines in order to define the microbiological quality of some food categories, indicates in 6 Log CFU/g the microbiological limit for crustaceans raw meat. There are no specific studies about the microbiological quality of American lobsters' meat; studies are available on frozen crabs meat (ICMSF, 1998), indicating that counts below 5 Log CFU/g can be considered satisfactory, while those comprised between 5 and 6 Log CFU/g should be considered fairly satisfactory. Moreover, for fish products, the threshold value used to identify the end of the shelf-life is often 6 Log CFU/g (Olafsdottir *et al.,* 2005), even if the total bacterial count at the point of rejection can be around 7-8 Log CFU/g (Espe *et al.,* 2004). Generally, the values obtained from our raw meat samples were characterised by limited loads: the average was below 5 Log CFU/g, confirming a good hygienic quality, while only two samples resulted to exceed the 6 Log level (6.21 and 6.38 Log CFU/g) (Table 1). As expected, gills and guts were characterised by higher loads: in the intestine this is due to the presence of a great amount of bacteria which constitute the natural microbiota, while the gills were identified as the main point of access of the bacteria from the outside due to the peculiar anatomical structure that allows gaseous exchanges. These loads, in any case, do not represent a hygienic problem as the product is generally consumed after a thermal treatment. However, in order to avoid the cross contami-

Table 1. Mean bacterial counts, number of countable samples and number of samples under the 6 Log limit of meat, gills and gut of American lobsters.

		Mean±SD (no. of countable samples)	Detection limit of 6 Log level (no. of samples under the limit)
TVC 20°C	Meat	4.5±0.9 (n=16)	<2 (n=2)
	Gills	7.1±0.8 (n=12)	<2 (n=0)
	Gut	6.5±1.3 (n=16)	<2 (n=0)
Bioluminescent bacteria	Meat	3.1±0.9 (n=7)	<2 (n=8)
	Gills	4.4±1.6 (n=10)	<2 (n=2)
	Gut	4.3±1.5 (n=8)	<2 (n=5)
H_2S producing bacteria	Meat	2.4±1.4 (n=6)	<1 (n=9)
	Gills	4.0±1.4 (n=7)	<1 (n=3)
	Gut	3.3±1.4 (n=8)	<1 (n=5)
Enterobacteriaceae	Meat	(n=0)	<2 (n=15)
	Gills	3.6±1.3 (n=3)	<2 (n=9)
	Gut	3.7±2.0 (n=3)	<2 (n=10)

SD, standard deviation; TVC, total viable count.

nation between raw products during the preparation, the good manufacturing and hygienic practices should be always applied. Concerning the other parameters evaluated, all the counts of meat samples resulted low (average values of bioluminescent and H_2S producing bacteria were always below 4 Log CFU/g). Considering H_2S producing bacteria, typical spoiler of fish products, although the loads detected in meat samples do not reach high levels, they should be carefully considered, due to their potential replication during the incidental further storage at low temperatures. For all the parameters, higher values were detected in gills and gut. Considering *Enterobacteriaceae* and fluorescent pseudomonads, all the samples were below the detection limit (2 Log CFU/g). The absence of detectable counts of fluorescent pseudomonads was unexpected, as these microorganisms are common contaminants of marine fishes (Huss, 1995).

In order to understand the ecology of the strains grown on the plates of the total viable count of meat samples, some phenotypic traits were considered: the 43.1% resulted to ferment glucose while the remaining 56.9% to be oxidative. Considering the ability to grow at different temperatures, the psychrotrophic nature of the microbiota was evidenced, even if the ability to adapt to higher temperatures was also observed. In fact, all the isolates grew at refrigerated temperatures (5°C), the 46.6% of the isolates was able to grow at 30°C while only the 3.4% resulted to be able to grow at 37°C and none of the isolates was able to grow at 42°C. Taking in account the salt requirement, the 82.8% of isolates resulted to be able to grow at 0.5% of salinity, while only the 24.1% at 0%, evidencing that the main lobster bacterial population is composed by halotolerant and halophilic microorganisms. From the 60 isolates randomly picked from the plates of meat samples, 2 were lost due to their short surviving *in vitro*. All the isolates were Gram Negative (Table 2).

The isolates clustered mostly (43.1%) with type strains from *Pseudoalteromonas* spp.: this is one of the largest genera within the *Gammaproteobacteria* and currently comprises more than 30 species (Ivanova *et al.,* 2004). These microorganisms are characterised by a widespread distribution across many marine habitats (*e.g.*, seawater, rocks, macroalgae and marine animals) and are able to survive in poor nutrient substrates producing a varied range of metabolites and enzymes (Ivanova *et al.,* 2003). The phylogenetic structure of this group indicates that the non-pigmented species are strictly related among them and constitute the major cluster of the genus. In our case, 25 isolates were identified as *Pseudoalteromonas* spp.: 20 isolates (80%) resulted to be closely related to the species *Ps.*

Table 2. Biomolecular identification of bacterial isolates from American lobsters meat and similarity rate.

Subject code	Isolate number	Closest relative in database and similarity rate
A	1	*Pseudoalteromonas paragorgicola/elyakovi/distincta/nigrifaciens* (99%)
A	2	*Pseudoalteromonas paragorgicola/elyakovi/distincta/nigrifaciens* (99%)
A	3	*Psychrobacter submarinus* (99%)
A	4	*Pseudoalteromonas paragorgicola/elyakovi/distincta/nigrifaciens* (99%)
A	5	*Pseudoalteromonas paragorgicola/elyakovi/distincta/nigrifaciens* (99%)
B	6	*Photobacterium kishitani/piscicola* (99%)
B	7	*Photobacterium kishitani* (99%)
B	8	*Photobacterium kishitani* (99%)
B	9	*Photobacterium kishitani/aquimaris* (99%)
B	10	*Vibrio rumoiensis* (98%)
C	11	*Psychrobacter pulmonis* (99%)
C	12	*Psychrobacter pulmonis* (100%)
C	13	*Pseudoalteromonas aliena* (99%)
C	15	*Vibrio rumoiensis/litoralis* (98%)
D	16	*Pseudoalteromonas paragorgicola/elyakovi/distincta/nigrifaciens* (99%)
D	17	*Vibrio lentus* (100%)
D	18	*Pseudoalteromonas marina* (98%)
D	19	*Pseudoalteromonas translucida* (99%)
D	20	*Psychrobacter piscatorii/nivimaris* (99%)
E	21	*Photobacterium frigidiphilum* (99%)
E	22	*Photobacterium frigidiphilum* (99%)
E	23	*Photobacterium frigidiphilum* (99%)
E	24	*Photobacterium frigidiphilum* (99%)
E	25	*Photobacterium frigidiphilum* (99%)
F	26	*Pseudoalteromonas paragorgicola/elyakovi/distincta/nigrifaciens* (99%)
F	27	*Pseudomonas arctica/Alteromonas fuliginea* (99%)
F	28	*Psychromonas arctica* (99%)
F	29	*Psychromonas arctica* (99%)
F	30	*Pseudoalteromonas paragorgicola/elyakovi/distincta/nigrifaciens* (99%)
G	31	*Psychromonas arctica* (99%)
G	32	*Psychromonas arctica* (99%)
G	33	*Pseudoalteromonas marina* (98%)
G	34	*Polaribacter sejongensis/butkevichii* (98%)
G	35	*Psychromonas arctica* (99%)
H	36	*Pseudoalteromonas paragorgicola/elyakovi/distincta/nigrifaciens* (100%)
H	37	*Pseudoalteromonas paragorgicola/elyakovi/distincta/nigrifaciens* (100%)
H	38	*Pseudoalteromonas paragorgicola/elyakovi/distincta/nigrifaciens* (100%)
H	39	*Pseudoalteromonas paragorgicola/elyakovi/distincta/nigrifaciens* (100%)
H	40	*Pseudoalteromonas paragorgicola/elyakovi/distincta/nigrifaciens* (100%)
I	41	*Alivibrio logei/salmonicida* (100%)
I	42	*Pseudoalteromonas paragorgicola/elyakovi/distincta/nigrifaciens* (100%)
I	43	*Pseudoalteromonas paragorgicola/elyakovi/distincta/nigrifaciens* (100%)
I	44	*Pseudoalteromonas paragorgicola/elyakovi/distincta/nigrifaciens* (100%)
I	45	*Pseudoalteromonas traslucida* (99%)
L	46	*Pseudomonas sabulinigri* (98%)
L	47	*Psychromonas arctica* (99%)
L	49	*Shewanella vesiculosa/livingstonensis* (98%)
L	50	*Pseudoalteromonas paragorgicola/elyakovi/distincta/nigrifaciens* (100%)
M	51	*Pseudoalteromonas paragorgicola/elyakovi/distincta/nigrifaciens* (100%)
M	52	*Shewanella arctica/frigidimarina* (100%)
M	53	*Pseudoalteromonas paragorgicola/elyakovi/distincta/nigrifaciens* (100%)
M	54	*Pseudoalteromonas paragorgicola/elyakovi/distincta/nigrifaciens* (100%)
M	55	*Pseudoalteromonas paragorgicola/elyakovi/distincta/nigrifaciens* (100%)
N	56	*Photobacterium kishitani/piscicola* (99%)
N	57	*Photobacterium aquimaris* (98%)
N	58	*Photobacterium kishitani/piscicola* (99%)
N	59	*Photobacterium kishitani/piscicola* (99%)
N	60	*Photobacterium kishitani/piscicola* (99%)

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paragorgicola, *elyakovi*, *distincta* and *nigrifaciens*. Our sequences did not allow the distinction of one of these 4 species due to the high similarity existing among them. Two isolates resulted to be similar to *Ps. marina*, two as *Ps. translucida* and one as *Ps. aliena*, all often isolated from marine environment (Nam *et al.,* 2007; Ivanova *et al.,* 2002, 2004).

Fourteen colonies (24.1%) were identified as *Photobacterium* spp., genus that comprises 19 species, five of those containing luminous strains (*Ph. phosphoreum*, *Ph. kishitani*, *Ph. angustum*, *Ph. ganghwense, Ph. leiognathi*): all these bacteria are commonly present in marine environments and occur in seawater, surfaces and gut of marine animals, marine sediments and saline lake water, as well as in the organs of fishes (Baumann and Baumann, 1984; Dalgaard, 1995; Urbanczyk *et al.,* 2011). Five of the isolates resulted to be related to *Ph. frigidiphilum*, a psychrophilic and weakly halophilic strain, firstly isolated and identified from deep-sea sediments of Edison Seamount (Seo *et al.,* 2005). Seven other isolates showed to be close to *Ph. kishitani:* for two isolates the species was confirmed with both the alignment tools while for five of them the species indicated in Blast algorithm differed and resulted as *Ph. piscicola,* a recent new one (Figge *et al.,* 2014), not yet included in the LeBibi data bank. One strain was related to *Ph. aquimaris* and one resulted to be similar with the same score to both the species *Ph. kishitani* and *Ph. aquimaris*: according to Yoshizawa *et al*. (2009), who firstly identified the latter species, these two are recognised to be very closely related from a genetic point of view. All the six isolates recognised as *Psychromonas* spp. (10.3% of the whole) resulted to be close to *Ps. arctica,* firstly identified in 2002: these cold-adapted microorganisms could be isolated from permanently but also temporarily cold locations (*e.g.*, oceans, polar regions, high mountains, deep lakes), and are often associated with plants and fresh and cold blooded fishes and crustaceans (Groudieva *et al.,* 2003).

Four isolates clustered with the genus *Psychrobacter* spp. (6.9% of the whole): these microorganisms are usually halotolerant and psychrophilic and are linked to several different substrates like fish skin, gills and gut, sea water but also lamb carcasses (Bowman *et al.,* 1997b; Gonzalez *et al.,* 2000; Prieto *et al.,* 1992). Two of these isolates resulted to be related to *P. pulmonis*, the only species not related to marine environment as firstly isolated and identified in 2002 from a pure culture from lung clinical specimen of lambs. Another one resulted to be close to *P. submarinus*, firstly isolated from sea water (Romanenko *et al.,* 2002) and another one to *P. piscatorii/nivimaris*, species firstly isolated from a drain of a fish-processing plant (Yumoto *et al.,* 2010) and from samples of the Southern Ocean environment (Heuchert *et al.,* 2004). As shown by Yumoto *et al*. (2010), the two species *P. piscatorii* and *P. nivimaris* have a deep genetic relatedness. Three isolates were identified as *Vibrio* spp. (5.2%): one resulted to be related to *Vibrio lentus*, firstly isolated from Mediterranean Oysters (Macian *et al.,* 2001), and the other to *Vibrio rumoiensis*, firstly isolated from drain pool of a fish product processing plant (Yumoto *et al.,* 2013). The third isolate was close to *V. rumoiensis/litoralis*: these two species are considered strictly related, as observed by Nam *et al*. (2007). Some species of *Vibrio* (*Vibrio vulnificus* and *V. parahaemolyticus* in particular) are primary causes of human mortality and illness due to gastroenteritis or septicemia through consumption of raw or undercooked seafood or from wound infections (Constantin de Magny *et al*. 2009). The majority of *V. vulnificus* and *V. parahaemolyticus* illnesses are often related with shellfish consumption; nevertheless, these bacteria can also be found in fish, sediment, plankton and marine water: in our study we did not focus on the presence and isolation of pathogenic bacteria, such as *Vibrio* spp., thus the colonies identified were isolated from a non-selective medium used for the enumeration of total viable count, and not from a specific medium for the isolation of these bacteria.

Two strains were identified as *Shewanella* spp.: one resulted to be similar to the species *S. vesiculosa,* firstly isolated from marine sediments collected at Deception Island, and *S. livingstonensis*, firstly isolated from Antarctic coastal marine environments (Bozal *et al.,* 2002; 2009). The other strain was related to *Shewanella arctica/frigidimarina,* firstly isolated respectively from Arctic marine sediment in a fjord branch in Norway and from sea ice from Antarctic meromictic lakes (Bowman *et al.,* 1997a; Kim *et al.,* 2012).

Two strains were identified as *Pseudomonas* spp.: one as *Pseudomonas sabulinigri,* firstly isolated from black sand collected from Korea (Kim *et al.,* 2009) and one as *Pseudomonas arctica/Alteromonas fuliginea.*

The remaining two isolates were identified as *Alivibrio logei/salmonicida*, two psychrophilic species whose substantial similarities were highlighted by multigenic analysis (Ast *et al.,* 2009), and as *Polaribacter sejongensis/butkevichii* firstly isolated from Antarctic soil and from sea water collected in the Sea of Japan (Kim *et al.,* 2013; Nedashkovskaya *et al.,* 2005).

Keeping into account only unique identifications, total number of bacterial species was 68: the 13 unique species observed are nearly 19% of total number of species. Nevertheless, considering ambiguous identifications, an algorithm was build up and performed in SAS software (SAS Institute, Cary, NC, USA) by which, the estimates were calculated for 100,000 random possible species compositions of a sample. According with this simulation, the highest value found for number of bacterial species was 53, while the mean number was 37. Number of total bacterial species colonising lobster meat calculated according only unique identifications should be considered as an over-estimation: as a consequence, values coming from simulation are better estimates of such species richness.

The results of our identifications partially agree with those obtained by Gornik *et al*. (2011) in Norway lobster (*Nephrops norvegicus*) tail meat. In fact most of the microbial spoilage population found was composed by the same genera *Vibrio* spp., *Photobacterium* spp., *Pseudoalteromonas* spp., *Shewanella* spp. and *Psychrobacter* spp., but the relative frequencies were quite different. In particular, the most prevalent genus in American lobsters was *Pseudoalteromonas* spp., while only few *Vibrio* spp. were found, while in Norway lobsters, *Vibrio* spp. represented more than half of the isolates and only 14.6% belonged to the

Table 3. Lipolytic and proteolytic abilities of the fifty-eight isolates from American lobsters meat.

	Lipolytic activity (%)	Proteolytic activity (%)
Total of the colonies identified	75.5 (46/58)	69.3 (38/58)
Pseudoalteromonas spp.	100 (24/24)	100 (24/24)
Photobacterium spp.	50.0 (7/14)	35.7 (5/14)
Psychromonas spp.	83.3 (5/6)	16.7 (1/6)
Psychrobacter spp.	50.0 (2/4)	0 (0/4)
Vibrio spp.	66.7 (2/3)	33.3 (1/3)
Pseudomonas spp.	100 (2/2)	50 (1/2)
Shewanella spp.	100 (2/2)	100 (2/2)
Polaribacter spp.	0 (0/1)	0 (0/1)
Alivibrio spp.	0 (0/1)	0 (0/1)

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genus *Pseudoalteromonas*.

Considering the genera identified, a clear inhomogeneity in the samples distribution was observed with some of them showing a strict prevalence of *Pseudoalteromonas* spp. (*e.g*., subjects code A, D, H, I, M) and some others of *Photobacterium* spp. (B, E, N). As a result, a single subject is not reflecting the microbial population of all the conspecifics and consequently it is quite difficult to predict the development of the spoilage microbiota in this product. In any case, all the identified spoilage microorganisms were common natural marine bacteria, and no important post-harvest contamination by not halophilic bacteria, due to handling or cross contamination, was evidenced. All the genera, apart from *Vibrio* spp., are microbial spoilers of seafood (Dalgaard, 2000; Gram and Huss, 1996; Gram *et al.,* 1987; Huss, 1995; Shewan, 1977).

All the isolates were screened for their proteolytic and lipolytic ability (Table 3), showing the wide diffusion of metabolically active strains. In particular, all *Pseudoalteromonas* spp. isolates resulted to possess lipolytic abilities and also proteolytic capabilities. *Photobacterium* spp., *Psychrobacter* spp., *Vibrio* spp. and *Psychromonas* spp. showed better lipolytic activity than proteolytic. None of the isolates in this study showed the ability to produce H_2S: this finding is in agreement with the data obtained from fresh Norway lobsters (Gornik *et al.,* 2011) where all the isolates showed not to possess this capability.

Conclusions

To our knowledge, no specific studies have been conducted so far about the microbiological quality of American lobsters' meat in terms of spoilage microbial population. The genera isolated from meat samples, mostly characterised by marine *Pseudoalteromonas* spp. and *Photobacterium* spp., could be considered representative of the microbiota of not-surviving subjects, but our data could also reflect the microbial environment of live lobsters, transported and stored in similar conditions. The evaluation of not surviving lobsters is of particular concern as they could be an optimal substrate for the growth of spoilage bacteria. Our samples showed very limited microbial loads, suggesting the possibility to commercialise these subjects without particular quality faults for the consumers. The application of good hygienical practices, especially during the production of ready-to-eat lobsters-based products, and a proper ice storage could limit the possible replication of these microorganisms.

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