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Novel insights into *hákarl*: A deep dive into the microbiological and physico-chemical features of Iceland's traditional fermented shark

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ABSTRACT

Hákarl, the traditional Icelandic product obtained from the fermentation of Greenland shark (*Somniosus microcephalus*), represents a unique food item from both cultural and microbiological perspectives. This study investigated commercial samples of *hákarl* using an integrated approach, combining physico-chemical analyses, lipid and volatile profiling, metataxonomic sequencing, and microbial isolation. Results confirmed the alkaline nature of the product (pH ~8) and a water activity (a_w) of 0.96, sufficient to sustain an active and diverse microbial community. Lipid profiling revealed the predominance of monounsaturated fatty acids, with differences in PUFA and DHA levels between the analyzed producers, consistent with nutritionally favorable characteristics. Microbiological analysis highlighted bacterial communities dominated by Firmicutes, particularly Bacilli and Clostridia, with significant abundances of *Tissierella creatinini* and *Atopostipes suicloacalis*. Culture-dependent methods led to the isolation of *Carnobacterium antarcticum* cultures, which were subsequently characterized for their enzymatic activities. These findings suggest potential biotechnological applications of the isolates, especially in fermentation and aroma development. Volatile compound analysis identified thirteen VOCs including alcohols, aldehydes, ketones, phenols, sulfur- and nitrogen-containing compounds. Trimethylamine was the predominant metabolite responsible for the strong ammonia-like odor, followed by phenol and sulfur-containing compounds, which also contributed to the sensory profile. Overall, the results provide novel insights into the microbial ecology, physico-chemical traits, and volatile characteristics of *hákarl*, confirming its variability linked to artisanal production methods. The study emphasizes *hákarl*'s role as a reservoir of pro-technological microorganisms and advances current understanding of the factors influencing its safety, quality, and identity as traditional fermented food.

1. Introduction

Iceland harbors a rich and largely undiscovered biodiversity shaped over millennia by the island's distinct and unique environmental conditions. As a result, the populations inhabiting Iceland have long adapted to exploit its natural resources to survive and thrive in both its harsh and captivating surroundings.

Iceland's cultural bond with the sea is central to its heritage, shaped in part by the prominence of the commercial fisheries sector. Despite a decline in consumption, seafood remains a staple of the Icelandic diet (Jones et al., 2025). According to the Government of Iceland, key fish species vital to the Icelandic economy include the Atlantic cod (*Gadus morhua*), haddock (*Melanogrammus aeglefinus*), saithe (*Pollachius virens*), ling (*Molva molva*), tusk (*Brosme brosme*), and whiting (*Merlangius merlangus*). Moreover, the cartilaginous fishes, considered "ancient species" for their limited evolutionary change over millions of years, comprise two modern groups: chimeras and elasmobranchs. Within the latter group, the Greenland shark (*Somniosus microcephalus*) is a slow-growing species that can reach a length of up to 7 m and feeds on nearly any available prey. While the Greenland shark can be found throughout

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Iceland, it is more commonly located in the colder northern waters. In the 18th century, this shark species was heavily exploited, with shark liver oil becoming one of Iceland's most significant exports, particularly as a fuel for streetlamps. Today, the shark is still caught in small quantities, primarily for the domestic market, where its liver oil and specially cured meat, namely *hákarl*, remain an important part of Icelandic culinary tradition, especially during festive occasions (Skåra et al., 2015).

According to Skåra et al. (2015), the exact origins of *hákarl* remain unclear, particularly whether the sharks were intentionally hunted or scavenged after washing ashore. Of note, eating of fresh Greenland shark is considered hazardous due to the presence urea and trimethylamine-N-oxide (TMAO) (Jensen et al., 2023).

Traditionally, *hákarl* was made by cutting the shark meat into chunks and burying them in gravel pits near the coast, covered with natural materials such as stones, seaweed, or turf. These pits were periodically submerged by seawater during high tides. In contrast, modern processing uses sealed containers that allow liquid runoff during fermentation, which typically lasts between 3 and 6 weeks depending on climate conditions. After this stage, the meat is dried in open-air sheds for several additional weeks or months. Nowadays, *hákarl* is also produced on an industrial scale, posing new challenges for the food industry, including the reduction of TMAO, shortening of the fermentation period, and standardization of the microbial community through the potential use of autochthonous starter cultures (Jensen et al., 2023).

In both traditional and modern techniques, the activity of the natural microbiota transforms the toxic flesh into a safe edible product with extended shelf life. *Hákarl* has a soft texture, a pungent ammonia-rich smell, and a pronounced fishy flavor.

Due to its distinctive production process, shaped by both tradition and environmental factors, *hákarl* serves as a unique reservoir of microbial diversity, harboring viable microbial species that have long adapted to extreme biological niches. To the authors' knowledge, only a few papers have described the microbiota of *hákarl*, where the dominant taxon was *Tissierella*, followed by *Oceanobacillus*, *Pseudomonas*, *Atopostipes*, and *Abyssivirga* (Osimani et al., 2019; Jensen et al., 2023). However, as noted by Jensen et al. (2023), the manufacturing of fermented shark remains an artisanal practice, with individual producers applying distinct methods passed down through generations. Consequently, there is no standardized protocol for the preparation of ready-to-eat *hákarl*. This leads to variability in the microbiological, chemical, and nutritional properties of the final product, which largely depend on the quality of the raw shark flesh and the specific techniques employed during processing. Therefore, consistent product characterization remains a challenge (Jensen et al., 2023). For this reason, the present study focuses on commercially available ready-to-eat *hákarl* rather than on process monitoring, aiming to characterize the microbial, physico-chemical, and volatile features of the final product as marketed and consumed, while accounting for producer- and batch-related variability inherent to this artisanal fermentation.

Moreover, to the best of the authors' knowledge, no microbial isolates from *hákarl* have yet been obtained and characterized. This makes *hákarl* a potentially valuable source of pro-technological microorganisms for the food industry. In this study, commercial samples of *hákarl* collected in Iceland were analyzed using both culture-dependent methods (viable cell counting) and a culture-independent approach (metataxonomic analysis) to investigate the native microbiota. To identify potential starter or adjunct cultures with desirable technological properties, 37 *Carnobacterium* cultures isolated from the samples were selected and assessed for some key enzymatic activities.

The aroma and chemical features of *hákarl* remain largely unexplored. Hence, to fill this research gap, volatile organic compounds (VOCs) were also characterized using headspace solid-phase microextraction coupled with gas chromatography–mass spectrometry (HS-SPME-GC/MS), and the fatty acid profile was established.

2. Materials and methods

2.1. Acquisition of specimens

Ready-to-eat *hákarl*, packaged in sealed plastic boxes containing 100 g of fish and sold under refrigerated conditions, was purchased from local retailers in southwest Iceland in June 2023. Samples were not subjected to any sterilization or terminal heat treatment and were sourced from two producers (HP1 and HP2), with two batches (B1 and B2) from each producer, purchased in duplicate. According to product labels, no ingredients other than fish flesh were used. Detailed information regarding the specific fermentation and drying conditions applied by the producers (e.g., duration, temperature, or environmental parameters) was not available, as the samples were obtained as commercial ready-to-eat products and such information is not disclosed on product labels. Given the artisanal and non-standardized nature of *hákarl* production, samples were therefore compared based on producer and batch as sources of variability in the final product rather than on defined processing parameters.

To preserve integrity, all collected samples were maintained at less than 4 °C and analyzed within 7 days.

2.2. Physico-chemical analyses

The pH of the samples was determined with a FiveEasy™ FE20 pH meter (Mettler-Toledo, Milan, Italy) fitted with an FC400B double-junction glass electrode (Hanna Instruments, Padova, Italy), in accordance with the MFHPB-03 protocol (Health and Food Products Branch, Government of Canada). Measurements were taken by immersing the electrode directly into the sample matrix.

Water activity (a_w) was assessed using an AquaLab 4TE analyzer (Meter Group, München, Germany), following the guidelines of ISO 18787:2017.

For each physico-chemical parameter evaluated, values were presented as the mean ± standard deviation based on two independent determinations.

2.3. Extraction of total lipids and fatty acid analysis

Fat extraction from *hákarl* samples was performed using a Soxhlet B-811 extractor (Buchi, Flawil, Switzerland), with 5 g of sample treated with 100 mL of n-hexane for 6 h at 70 °C. Fatty acid methyl esters (FAMEs) were prepared from 20 mg aliquots by alkaline transmethylation with 2 M KOH in methanol. The resulting FAMEs were analyzed by GC-FID (Trace 1300, Thermo Fisher Scientific, USA) following the procedure of Cardinali et al. (2025), using an Rt-2560 fused silica column (100 m × 0.25 mm i.d., 0.2 μm; Restek, USA). Identification was carried out with a C8–C24 FAME standard mix (Merck, Germany), and chromatograms were processed using Chromeleon 7.2.10. All analyses were performed in duplicate, and fatty acid composition was expressed as the relative percentage of total fatty acids.

2.4. Viable counts

To determine viable counts, 10 g of sample was blended with 90 mL of sterile peptone water (1 g L^{-1}). Homogenization was carried out for 2 min at 260 rpm using a Stomacher 400 Circulator (International PBI, Milan, Italy). Serial ten-fold dilutions were then prepared in maximum recovery diluent, and enumeration of microbial groups was performed following the procedures outlined in Table 1.

2.5. DNA extraction, amplicon-based sequencing, and metataxonomic analysis

Microbial DNA was isolated directly from *hákarl* samples using the PowerFood™ Microbial DNA Isolation Kit (Mo Bio Laboratories,

Table 1
Microbial counts growth media and incubation conditions.

Microorganisms	Growth medium	Supplement	Incubation temperature	Incubation time	Incubation conditions	Reference methods
Total aerobic mesophilic count	Plate Count Agar (VWR Prolabo Chemicals, Leuven, Belgium)	n.a.	30 °C	72 h	Aerobiosis	UNI EN ISO 4833-1:2022
Yeasts and molds	Dichloran Rose Bengal Chloramphenicol Agar (VWR Prolabo Chemicals)	n.a.	25 °C	5 days	Aerobiosis	ISO 21527-1:2008 applicable to products with $a_w > 0.95$
Coagulase-positive staphylococci	Baird-Parker Agar (VWR Prolabo Chemicals)	Rabbit Plasma Fibrinogen (BP-RPF) (VWR Prolabo Chemicals)	37 °C	48 h	Aerobiosis	UNI EN ISO 6888-2:2023
Coagulase-negative staphylococci	Mannitol Salt Agar (VWR Prolabo Chemicals)	n.a.	37 °C	48–72 h	Aerobiosis	–
Escherichia coli	REBECCA™ Agar (bioMérieux, Marcy-l'Étoile, France)	EB supplement (bioMérieux)	37 °C	24 h	Aerobiosis	AFNOR AES 10/06-01/08
Enterobacteriaceae	REBECCA™ Agar (bioMérieux)	EB supplement (bioMérieux)	37 °C	24 h	Aerobiosis	AES 10/07-01/08
Enterococci	Slanetz-Bartley Agar (VWR Prolabo Chemicals)	n.a.	35 °C	48 h	Aerobiosis	–
Sulfite-reducing bacteria	Iron Sulphite Agar (VWR Prolabo Chemicals)	n.a.	37 °C	48 h	Anaerobiosis using AnaeroGen™ system (Oxoid, Basingstoke, UK)	ISO 15213-1:2023
Clostridium perfringens	Iron Sulphite Agar (VWR Prolabo Chemicals)	400 mg L^{-1} d-cycloserine (VWR Prolabo Chemicals)	37 °C	18 h	Anaerobiosis using AnaeroGen™ system (Oxoid)	UNI EN ISO 7937:2005
Pseudomonas spp.	Penicillin Pimaricin Agar (VWR Prolabo Chemicals)	n.a.	25 °C	48 h	Aerobiosis	–
Presumptive lactic acid bacteria	De Man, Rogosa and Sharpe (MRS) Agar (VWR Prolabo Chemicals)	n.a.	30 °C	72 h	Aerobiosis	–
Arcobacter spp.[a]	Tryptic Soy Agar (VWR Prolabo Chemicals)	5% defibrinated sheep blood (VWR Prolabo Chemicals)	30 °C	72 h	Microaerophilic conditions using CampyGen™ system (Oxoid)	–

n.a. not added.

[a] For this microorganism, 10 g of sample were diluted 1:10 in *Arcobacter* enrichment broth supplemented with 8 mg L^{-1} cefoperazone, 10 mg L^{-1} amphotericin B, and 4 mg L^{-1} teicoplanin (ThermoScientific, Waltham, MA, USA), and incubated under microaerophilic conditions (CampyGen™, Oxoid, UK) at 30 °C for 48 h. The enriched broth was then subjected to passive membrane filtration and plated on Tryptic Soy Agar supplemented with 5% defibrinated sheep blood. Plates were incubated for 72 h under the same conditions (Gabucci et al., 2023).

Carlsbad, USA). For each sample, 1.5 mL of homogenate (10^{-1} dilution) was centrifuged to obtain a pellet, which was then processed following the kit protocol. DNA quality was evaluated with a NanoDrop spectrophotometer (Thermo Scientific, Milan, Italy).

The V3–V4 region of the bacterial 16S rRNA gene was amplified with primers described by Klindworth et al. (2013). Duplicate PCR products were purified using the Agencourt AMPure kit (Beckman Coulter, Milan, Italy), and sequencing adapters were ligated with the Nextera XT Library Preparation Kit (Illumina, San Diego, CA, USA). Sequencing was performed on the Illumina MiSeq platform with V3 chemistry, generating 2 × 250 bp paired-end reads. Base calling and barcode demultiplexing were carried out with MiSeq Control Software v2.3.0.3, RTA v1.18.42.0, and CASAVA v1.8.2.

Raw reads were processed in QIIME2 (Bolyen et al., 2019): primers were removed with Cutadapt, low-quality reads were trimmed, chimeras were filtered out, and paired-end reads were merged using the dada2 denoise-paired plugin (Callahan et al., 2016). Taxonomic assignment was performed with the QIIME2 feature-classifier against the Greengenes2 16S rRNA reference database.

Diversity analyses were conducted in MicrobiomeAnalyst (Chong et al., 2020). Alpha diversity indices and beta diversity (Bray–Curtis dissimilarity) were computed, and differences between producers were tested with the non-parametric Mann–Whitney *U* test.

Sequencing data are publicly available in the NCBI Sequence Read Archive under BioProject accession number PRJNA1256307.

2.6. Analysis of volatile compounds

Volatile organic compounds (VOCs) were analyzed by placing ~1 g of *hákarl* into 15 mL vials sealed with silicone/PTFE caps. Headspace SPME coupled with GC–MS (Clarus 600, Perkin Elmer, Italy) was used, employing a DVB/CAR/PDMS fiber (50/30 μm, Supelco, USA) and a TG-WAXMS column (30 m × 0.25 mm, 0.25 μm; Thermo Fisher Scientific). The extraction and chromatographic conditions followed the method described by Cardinali et al. (2025). VOCs identification was based on comparison of mass spectra and linear retention indices, calculated using a C7–C30 n-alkane series, with entries in the NIST online database. Each sample was run in duplicate, and compound abundances were expressed as relative percentages.

2.7. Isolation and characterization of viable microorganisms

2.7.1. Isolation and identification

Colonies grown on Plate Count Agar (VWR) at 30 °C were randomly selected and re-streaked on the same medium to confirm purity. Pure isolates were stored at −80 °C until analysis. Prior to DNA extraction, frozen cultures were thawed and sub-cultured twice on PCA (VWR) at 30 °C for 48–72 h. DNA was extracted according to the protocol described by Osimani et al. (2015). DNA quality and concentration were measured with a NanoDrop ND 1000 spectrophotometer (Thermo Fisher Scientific) and then adjusted to 100 ng μL^{-1}. The standardized DNA was amplified by PCR using a MyCycler Thermal Cycler (Bio-Rad, Hercules, CA, USA) and analyzed by gel electrophoresis, following Osimani et al. (2015). Amplicons were purified and sequenced by Genewiz (Takaley, UK). The sequences obtained were compared with type strain 16S rRNA gene sequences in the GenBank database (http://www.ncbi.nlm.nih.gov/) using BLAST (Basic Local Alignment Search Tool). Accession numbers for the isolates were subsequently obtained upon sequence submission to GenBank.

2.7.2. Semi-quantitative assessment of enzymatic activity

The semi-quantitative API® ZYM micromethod (bioMérieux, Marcy l'Etoile, France) was employed to evaluate the key enzymatic activities of the selected lactic acid bacteria, following the manufacturer's protocol. Each API® ZYM strip consists of 20 cupules containing dehydrated synthetic substrates, which are rehydrated by inoculation with a water suspension of the test microorganism. These cupules are designed to assess the enzymatic activities reported in Fig. 5.

Color development, indicating the formation of metabolic end-products during incubation, was visualized after adding the appropriate reagents. For each isolate, enzymatic activity was scored on a scale from 0 to 5, with 0 indicating no reaction, 5 representing maximum intensity, and intermediate values (1–4) corresponding to increasing levels of activity. Scores of 3, 4, or 5 were considered positive reactions (Cardinali et al., 2025).

2.8. Statistical analysis

Statistical differences among samples were evaluated using one-way analysis of variance (ANOVA) followed by Tukey–Kramer's Honest Significant Difference (HSD) test at a significance level of 0.05. All analyses were conducted using JMP software, version 11.0.0 (SAS Institute Inc., Cary, NC).

3. Results and discussion

In the present study, the analysis of multiple batches from different producers was intentionally adopted to capture the intrinsic variability of ready-to-eat *hákarl*, a product obtained through non-standardized artisanal practices, and to assess the consistency of dominant microbial taxa and functional traits persisting in the final product. While studies conducted during the fermentation process are essential to elucidate microbial succession, investigations on ready-to-eat products provide complementary information directly relevant to consumer exposure, product safety, sensory characteristics, and the identification of viable microorganisms with potential technological applications.

3.1. Physico-chemical analyses

Physico-chemical results obtained from the analyzed samples are reported in Table 2. In more detail, all samples exhibited the same a_w value of 0.96. Notably, this a_w level is sufficient to support the growth of most microorganisms, highlighting that *hákarl* constitutes an active and microbiologically rich biological matrix. To the author's knowledge, no other studies have reported the water activity of *hákarl*, and therefore no direct comparison with previously published data is currently possible. However, it is likely that the detected a_w values are the result of the fermentation and drying steps applied during *hákarl* manufacturing.

Regarding pH, the alkaline nature of *hákarl* was confirmed, with measured values averaging around 8. Although only a limited number of studies on *hákarl* are available in the scientific literature, the present findings are consistent with those reported by Osimani et al. (2019) and Jensen et al. (2023), who observed comparable pH levels in ready-to-eat *hákarl* samples and in samples at the end of the drying process, respectively. According to Samyal (2022), alkaline fermentation involves the microbial breakdown of substrate proteins into peptides, free amino acids, and ammonia. This process is typically accompanied by a substantial rise in amino acids such as methionine, cysteine, tyrosine, leucine, isoleucine, phenylalanine, and lysine.

Table 2

Results of physico-chemical parameters of the analyzed *hákarl* samples.

Producer	Batch	Sample	a_w	pH
1	1	HP1B1	0.96 ± 0.00	8.27 ± 0.00
	2	HP1B2	0.96 ± 0.00	8.06 ± 0.01
Overall mean			0.96 ± 0.00 ^{a}	8.16 ± 0.14 ^{a}
2	1	HP2B1	0.96 ± 0.00	8.28 ± 0.01
	2	HP2B2	0.96 ± 0.00	8.29 ± 0.00
Overall mean			0.96 ± 0.00 ^{a}	8.28 ± 0.01 ^{a}

Values are expressed as mean ± standard deviation.

For each parameter, overall means followed by different letters are significantly different ($P < 0.05$).

3.2. Total fatty acid profile

The total extracted lipid and fatty acid composition of ready-to-eat *hákarl* from two producers (HP1 and HP2) are shown in Table 3. Oleic acid and eicosenoic acid were the most abundant fatty acids in both products, together accounting for more than 50% of the total. As a result, monounsaturated fatty acids (MUFAs) represented the dominant group, with no significant differences observed between the two producers ($p > 0.05$). These results are characteristic of marine-derived lipids and are consistent with previous findings on the fatty acid profile of Greenland shark muscle (McMeans et al., 2012; McMeans et al., 2013; Xu et al., 2018). More variation was observed in polyunsaturated fatty acid (PUFA), which contributed 21.95% in HP1 and 15.11% in HP2. The lower PUFA levels in HP2 may be linked to lipid oxidation, potentially influenced by factors such as the choice of fermentation temperature and duration (Bekhit et al., 2018; Xu et al., 2018). However, this interpretation is not supported by direct measurements of lipid oxidation indicators (e.g., peroxide value or thiobarbituric acid reactive substances) and should therefore be regarded as a limitation of the present study.

Since oleic and linoleic acid levels did not differ significantly between the two producers, it is suggested that the fermentation process was at a similar stage in both cases (Xu et al., 2018). Additionally, *hákarl* microbiota may have encouraged the desaturation of stearic acid (C18:0) (Xu et al., 2018), which was below the detection limit ($<0.05\%$) in all analyzed samples. Notably, HP1 had significantly higher levels of

Table 3

Total Lipids and Fatty Acid Composition (weight % of total fatty acids) of the ready-to-eat *hákarl* from two producers.

Fatty acids (%)	HP1	HP2
Myristic (C14:0)	1.19 ± 0.1	1.84 ± 0.1
Pentadecanoic (C15:0)	0.18 ± 0.1	0.24 ± 0.1
Palmitic (C16:0)	15.39 ± 0.1^{a}	20.07 ± 0.7^{b}
Heneicosanoic (C21:0)	0.29 ± 0.1	0.22 ± 0.1
ΣSFA	17.05	22.38
Palmitoleic (C16:1)	6.89 ± 0.2	8.76 ± 0.5
Oleic (C18:1)	34.07 ± 0.9	36.39 ± 1.1
Eicosenoic (C20:1 c)	18.00 ± 0.4	16.18 ± 0.8
Nervonic (C24:1)	2.04 ± 0.1	1.18 ± 0.2
ΣMUFA	61.00	62.51
Linoleic (C18:2Δ9, 12)	1.32 ± 0.1	1.50 ± 0.3
Linolenic (C18:3Δ9,12, 15)	0.25 ± 0.1	0.28 ± 0.1
Cis-11, 14, 17-eicosatrienoic (C20:3)	7.35 ± 0.7	5.51 ± 0.6
Arachidonic (C20:4)	0.80 ± 0.2	0.47 ± 0.1
Cis,cis-docosadienoic (C22:2 c)	4.38 ± 0.6	3.24 ± 0.3
DHA (C22:6)	7.85 ± 0.5^{a}	4.12 ± 0.8^{b}
ΣPUFA	21.95	15.11
Σω6	9.48	7.48
Σω3	8.09	4.39
UFA/SFA	4.86	3.47
PUFA/SFA	1.29	0.68
ω3/ω6	0.85	0.58
ω6/ω3	1.17	1.70
Lipid (%)	21.02 ± 0.9^{a}	16.25 ± 0.7^{b}

SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; DHA, cis-4, 7, 10, 13, 16, 19-docosahexaenoic; Cm:n Δx; m = number of carbon atoms, n = number of double bonds, x = position of double bonds; Results represent means ± SD; Different small letters in the same row mean significant differences ($P < 0.05$).

docosahexaenoic acid (DHA) (7.85%) compared to HP2 (4.12%). These differences are likely linked to variations in shark tissue and/or the fermentation process, which can significantly impact DHA levels (Bekhit et al., 2018). Nonetheless, samples from both producers showed favorable ω-6/ω-3 and ω-3/ω-6 ratios (<4 and >0.2, respectively), consistent with nutritional recommendations (Simopoulos, 2002). The ω-3/ω-6 ratio and DHA content are considered key indicators of lipid quality in seafood, showing healthy effects on the prevention of cardiovascular diseases and cancer (Nartea et al., 2023; Pauwels, 2011).

3.3. Viable counts

Viable count results are reported in Table 4. More specifically, among the microbial groups tested, only total mesophilic aerobes exhibited notable counts, with average values ranging from 3.32 to 4.80 log cfu g^{-1}. The detected values are in accordance with those detected in ready-to-eat *hákarl* by Osimani et al. (2019) and by Jensen et al. (2023) in *hákarl* samples analyzed at the end of the drying step. Of note, the group of total mesophilic aerobes usually include pro-technological, saprophytic, spoilage, and potentially pathogenic microorganisms. As already elucidated by Jensen et al. (2023), total mesophilic aerobe counts in *hákarl* have a rapid increase during the initial stage of fermentation, reaching a maximum after a few weeks (approximately 4), followed by a subsequent decrease during drying. Although psychrotrophic bacteria were not specifically quantified in this study, it is likely that the viable counts observed also included members of this group.

With respect to the low counts of *Pseudomonas* spp. (<2 log cfu g^{-1}), the values observed in this study are consistent with those reported by Osimani et al. (2019), who found levels between <1 and 1.6 log cfu g^{-1}, and with the counts recorded by Jensen et al. (2023) in *hákarl* at the end of drying (1–2 log cfu g^{-1}). Notably, like total mesophilic aerobes, Jensen et al. (2023) observed a progressive increase in *Pseudomonas* counts during the first four weeks of shark flesh fermentation, followed by a decline to below the detection limit.

The low counts of Enterobacteriaceae, *Escherichia coli*, yeasts, molds, sulfite-reducing anaerobes, *Clostridium perfringens*, and coagulase-positive staphylococci detected in this study are consistent with the findings of Osimani et al. (2019) and Jensen et al. (2023), confirming the inhibition of these microorganisms during fermentation.

As for the counts of presumptive lactic acid bacteria (including enterococci), the results obtained in the present study were somewhat contradictory. Specifically, counts <1 log cfu g^{-1} were observed in all the samples, irrespective of the producer or batch. However, the isolation campaign carried out on PCA plates used for enumerating total mesophilic aerobes yielded lactic acid bacteria isolates belonging to the *Carnobacterium* genus, which will be discussed in detail in the following paragraphs.

To the best of the authors' knowledge, *hákarl* has not previously been tested for *Arcobacter*. In the present study, this microorganism was absent in all analyzed samples (10 g of ready-to-eat product), contributing to current knowledge of the viable microbiota of this food matrix and supporting its safety of consumption.

3.4. Taxonomic diversity

A total of 37,596 reads were analyzed, with an average of approximately 4700 reads per sample. Alpha and beta diversity analyses revealed no statistically significant differences between the two producers (*p* > *0.05*), as indicated by the Chao1 richness index (Fig. 1, panel a), the Shannon diversity index (Fig. 1, panel b), and Bray–Curtis's dissimilarity visualized through Principal Coordinates Analysis (PCoA) (Fig. 2).

Fig. 3 shows the distribution of microbial communities at different taxonomic levels across the four *hákarl* samples (HP1B1, HP1B2, HP2B1, HP2B2). At the phylum level, Firmicutes dominated all samples (>95% of the relative frequency), with Proteobacteria present in minor

Table 4
Results of viable counting of bacteria and eumycetes in the analyzed *hákarl* samples.

Producer	Batch	Sample	Total mesophilic aerobes	*Pseudomonas* spp.	Enterobacteriaceae	Yeasts	Molds	Presumptive lactic acid bacteria	Coagulase-negative staphylococci	Enterococci	*Arcobacter*	*Escherichia coli*	Sulfite-reducing anaerobes	*Clostridium perfringens*	Coagulase-positive staphylococci
1	1	HP1B1	5.09 ± 0.05	<2	<1	<1	<1	<1	<1	<1	abs. 10 g	<1	<1	<1	<1
	2	HP1B2	3.19 ± 0.01	<2	<1	<1	<1	<1	<1	<1	abs. 10 g	<1	<1	<1	<1
Overall mean			4.80 ± 0.05^{a}	<2	<1	<1	<1	<1	<1	<1	abs. 10 g	<1	<1	<1	<1
2	1	HP2B1	3.48 ± 0.05	<2	<1	<1	<1	<1	<1	<1	abs. 10 g	<1	<1	<1	<1
	2	HP2B2	3.07 ± 0.01	<2	<1	<1	<1	<1	<1	<1	abs. 10 g	<1	<1	<1	<1
Overall mean			3.32 ± 0.05^{b}	<2	<1	<1	<1	<1	<1	<1	abs. 10 g	<1	<1	<1	<1

Values are expressed as Log cfu g^{-1} ± standard deviation.

abs. absence.

For each microorganism or microbial group, overall means followed by different letters are significantly different ($P < 0.05$).

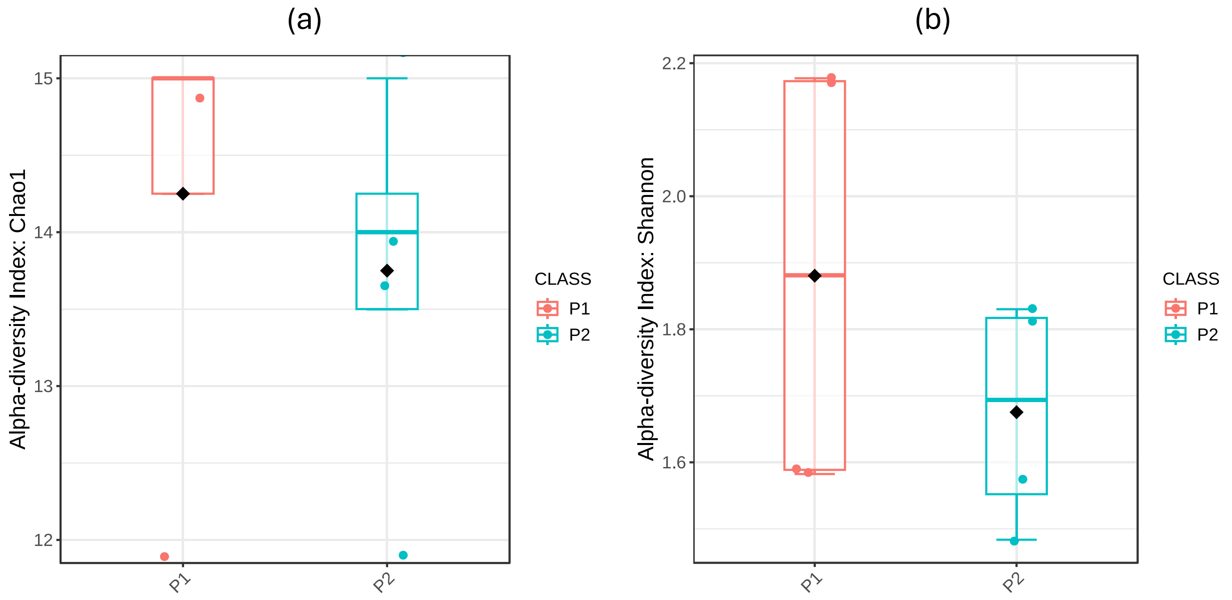

Fig. 1. Alpha diversity of bacterial communities in *hákarl* samples from two different producers (P1 and P2). (a) Chao1 richness index and (b) Shannon diversity index. No statistically significant differences were observed between producers (Mann–Whitney *U* test, $p > 0.05$).

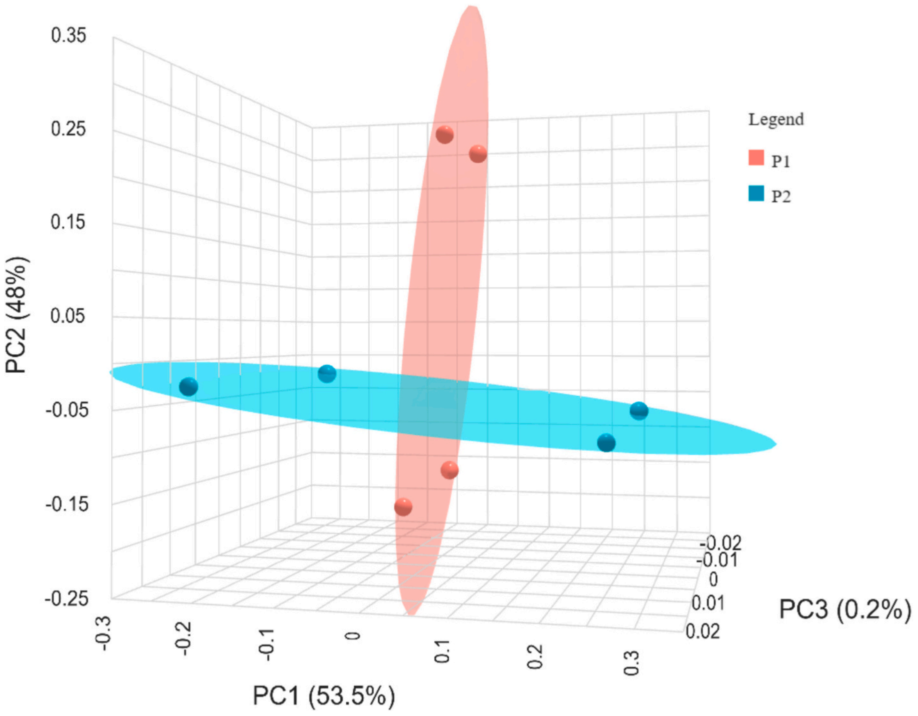

Fig. 2. Principal Coordinates Analysis (PCoA) based on Bray–Curtis dissimilarity of bacterial communities in *hákarl* samples from two producers (P1 and P2). No significant separation was observed between the two producers (Mann–Whitney *U* test, $p > 0.05$).

proportions (Fig. 3, panel a). At the class level, the bacterial community was primarily composed of Bacilli and Clostridia, with Gammaproteobacteria present at lower relative abundances (<4%), particularly in samples from producer 1 (Fig. 3, panel b). At the order level, a more detailed taxonomic resolution revealed that Bacillales, Tissierellales, and Lactobacillales were the most abundant orders in all samples. Samples HP1B2 and HP2B1 showed similar relative abundances of Clostridiales (∼4%) and Erysipelotrichales (∼2%), whereas Pseudomonadales were more prevalent in samples of producer 1. Notably, sample HP1B2 showed a significant presence of Lachnospirales (∼9%) (Fig. 3, panel c). At the family level (Fig. 3, panel d), Tissierellaceae and Amphibacillaceae were the most abundant taxa across all samples. Together, these two families accounted for more than 70% of the total relative abundance in each sample. The family Carnobacteriaceae was also detected in all samples, with relative abundances ranging from 4.10% to 20.19%. In addition, samples HP1B2 and HP2B1 showed the presence of Clostridiaceae and Erysipelotrichaceae, with relative abundances between 1.35% and 4.56%. Genus-level classification (Fig. 3, panel e) revealed the consistent presence of *Tissierella* and *Atopostipes* across all samples. The genus *Proteiniclasticum* was detected exclusively in samples HP1B2 and HP2B1.

The bacterial distribution of *hákarl* samples is presented in Fig. 4 and

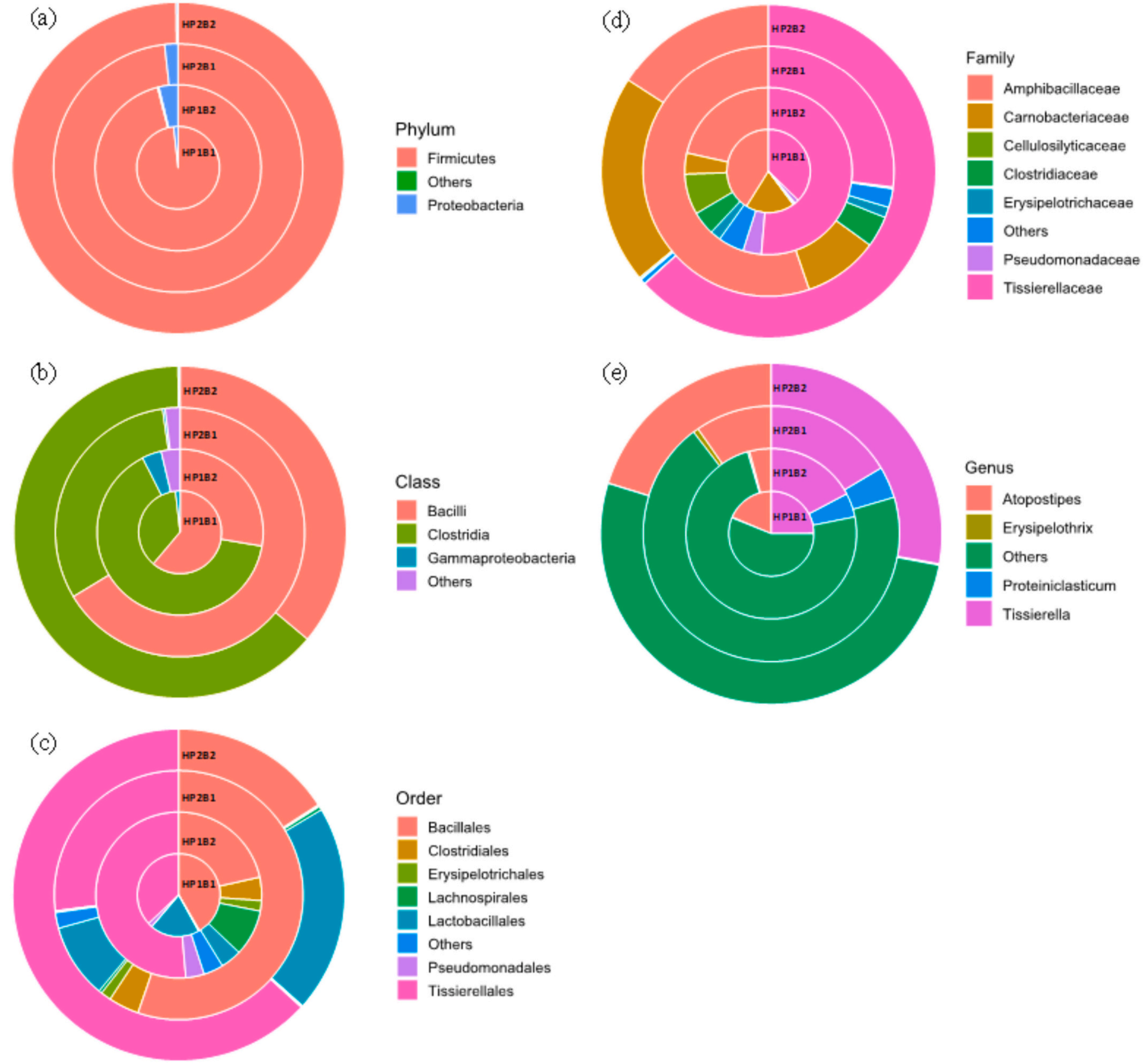

Fig. 3. Taxonomic composition of bacterial communities in *hákarl* samples (HP1B1, HP1B2, HP2B1, HP2B2) at different taxonomic levels. (a) Phylum level; (b) Class-level; (c) Order-level; (d) Family-level; (e) Genus-level.

Supplementary Table 1. Amphibacillaceae and Tissierellaceae were consistently detected across all samples, with relative abundances ranging from 7.86% to 55.25%. In more detail, the family Amphibacillaceae comprises halophilic bacteria capable of thriving in saline environments, including seafood (Miliotis et al., 2024). This family includes the genera *Tigheibacillus*, *Virgibacillus*, and *Oceanobacillus* (Miliotis et al., 2024). Notably, *Oceanobacillus* was previously detected in ready-to-eat *hákarl* by Osimani et al. (2019), suggesting that this food matrix may serve as a biological niche for Amphibacillaceae.

Tissierellaceae have previously been identified as a major bacterial family in *hákarl*, with the genus *Tissierella* reported by Osimani et al. (2019) and Jensen et al. (2023). *Tissierella* was also identified by Jang et al. (2017) as a key microorganism in alkaline-fermented skate, and its presence has been documented in fermented *Engraulidae*, *Decapterus* (*kusaya*), *Raja pulchra*, and *Raja kenojel* (*hongeo*) (Belleggia and Osimani, 2023), reinforcing its pivotal role in fermented fish products. *Tissierella* comprises anaerobic bacteria capable of degrading proteins and/or glucose, producing volatile fatty acids such as acetic, butyric, and propionic acids, along with carbon dioxide as metabolic end-products (Chen et al., 2018). Interestingly, *Tissierella* species are adapted to urea- and ammonia-rich environments, suggesting their ability to use such compounds, and potentially the high trimethylamine amounts in ready-to-eat *hákarl*, as carbon or nitrogen sources (Jensen et al., 2023). In the present study, *Tissierella creatinini* was particularly abundant, with relative frequencies ranging from 16.40% to 27.83%. This species was first described by Farrow et al. (1995). According to Alauzet et al. (2014), *T. creatinini* grows at temperatures between 20 and 39 °C, with an optimum at 37 °C; however, little is known about the biological role of this Gram-positive, rod-shaped bacterium in food.

Atopostipes suicloacalis was also detected in all *hákarl* samples, with relative frequencies ranging from 4.10% to 20.19%. This genus of Gram-positive facultatively anaerobic bacteria belonging to Carnobacteriaceae

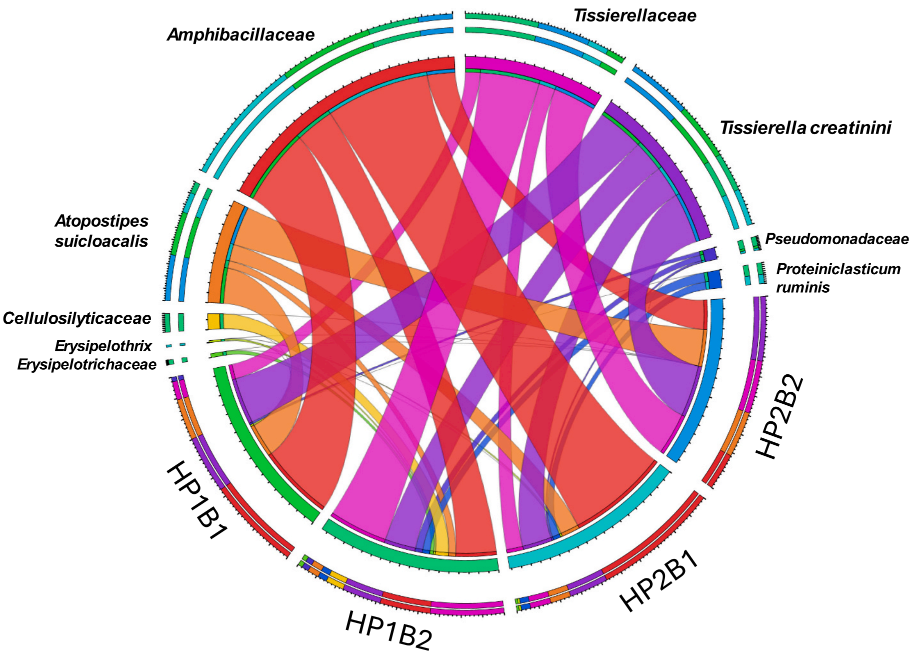

Fig. 4. Circular ideogram showing the distribution of bacterial Amplicon Sequence Variants (ASVs) across the *hákarl* samples. ASVs and samples are connected by ribbons, whose thickness is proportional to the abundance of ASVs in the connected samples. The outer circle displays the proportion of each ASV in each sample and vice versa. Only taxa annotated at a resolution higher than the order level and showing relative abundance values greater than 0.5% in at least two samples are included.

was previously identified by Jensen et al. (2023) as a major bacterial group in *hákarl* at the end of drying. Additionally, *Atopostipes* has been reported in the microbiota of fermented fish such as *Raja pulchra*, *hongeo*, and low-salt shrimp paste (*terasi*) (Helmi et al., 2022; Belleggia and Osimani, 2023). *A. suicloacalis* grows optimally between 28 and 30 °C and does not grow above 32 °C. Its glucose metabolism yields lactate, acetate, and formate as end products (Cotta et al., 2004). This bacterium is often found in alkaline environments rich in urea and ammonia, such as urine-derived fertilizers, and exhibits traits indicative of its ability to metabolize these compounds. As noted by Jensen et al. (2023), such adaptations may allow the *Atopostipes* detected in *hákarl* to utilize urea as well as ammonia as sources of nitrogen.

Samples HP1B2 and HP2B1 showed the common presence of *Proteiniclasticum ruminis* (~4%). Although detected at low frequency, this taxon has also been detected by Osimani et al. (2019) in ready-to eat *hákarl*. Remarkably, this strictly anaerobic species, originally isolated from the rumen of yaks, exhibits strong proteolytic activity (Zhang et al., 2010), which may play a role in the breakdown of proteins during *hákarl* fermentation.

In the analyzed samples, Cellulosilyticaceae were found exclusively in sample HP1B2, representing 7.86% of the relative abundance. To date, no studies on *hákarl* fermentation have reported the presence of bacteria from this family. As reported by Dong (2015), Cellulosilyticaceae species are slightly curved, Gram-positive rods forming terminal spores, growing optimally at 40 °C under strict anaerobic conditions. They ferment cellulose and xylan to produce acetate, ethanol, and formate. The Cellulosilyticaceae family includes genera such as *Cellulosilyticum* and *Holtiella*, which remain poorly studied in the context of food microbiology. However, members of this family have been identified in the gut microbiota of Chinese soft-shelled turtles, suggesting their association with the intestine of aquatic animals (Zhou et al., 2025).

Samples from producer 1 contained higher levels of Pseudomonadaceae (1.69% in HP1B1 and 3.59% in HP1B2) than those from producer 2 (<0.5% in both HP2B1 and HP2B2). Previous studies on *hákarl* reported Pseudomonadaceae (and *Pseudomonas*) among the dominant bacterial groups (Osimani et al., 2019; Jensen et al., 2023). In contrast, their occurrence in the present study was limited, reinforcing the notion of high microbiota variability in *hákarl* (Jensen et al., 2023).

Minor taxa were occasionally observed, including Carnobacteriaceae, *Carnobacterium maltaromaticum*, Dysgonomonadaceae, *Erysipelothrix*, Erysipelotrichaceae, *Sporosarcina*, and Vagococcaceae. Among these taxa, Carnobacteriaceae and *Carnobacterium* merit further attention, as species of carnobacteria were isolated from PCA plates in this study. Hence, their relevance in the analyzed *hákarl* samples will be addressed in the following paragraph. Notably, *Erysipelothrix* and *Sporosarcina* have also been reported in *hákarl* by Osimani et al. (2019) and Jensen et al. (2023), respectively.

3.5. Isolation and characterization of carnobacteria

The isolation and characterization results of viable microorganisms grown on PCA plates are reported in Table 5. Remarkably, 37 out of the 39 isolates were assigned to the lactic acid bacteria genus *Carnobacterium*; moreover, 2 isolates of *Staphylococcus epidermidis* were also recovered. Among carnobacteria isolates, 1 culture belonged to the species *Carnobacterium mobile*, whereas 36 isolates belonged to *Carnobacterium antarcticum*.

Although MRS agar is considered the most suitable medium for enumerating and isolating lactic acid bacteria, in this study, the viable counts carried out using this growth medium were <1 log cfu g^{-1}, suggesting a somewhat contradictory picture of the microbial community. Such low counts may be explained by bacterial succession during *hákarl* fermentation, in which lactic acid bacteria are initially abundant but decline over time (Jensen et al., 2023). Furthermore, the recovery of lactic acid bacteria on PCA plates suggests that only a fraction of the colonies counted on this medium were actually lactic acid bacteria, with those present being better adapted to these cultural conditions rather than MRS.

While lactic acid bacteria are typically associated with acidification, acid production by carnobacteria can vary depending on strain and process parameters such as temperature and time (Edima et al., 2008). Given the alkaline nature of *hákarl*, the contribution of *Carnobacterium*

Table 5
Lactic acid bacteria cultures isolated from hákarl samples.

Isolate code	Closest relatives	Isolation source	% Identity[a]	Accession number
HP1A	*Carnobacterium antarcticum*	HP1B1 (1)	99.91%	NR_179901
HP1B	*Carnobacterium antarcticum*	HP1B1 (1)	99.38%	NR_179901
HP1C	*Carnobacterium antarcticum*	HP1B1 (1)	99.64%	NR_179901
HP1D	*Carnobacterium antarcticum*	HP1B1 (1)	99.91%	NR_179901
HP1E	*Carnobacterium antarcticum*	HP1B1 (1)	99.91%	NR_179901
HP1F	*Carnobacterium mobile*	HP1B1 (2)	99.55%	NR_040926
HP1G	*Carnobacterium antarcticum*	HP1B1 (2)	99.64%	NR_179901
HP1H	*Carnobacterium antarcticum*	HP1B1 (2)	99.46%	NR_179901
HP1I	*Carnobacterium antarcticum*	HP1B1 (2)	99.46%	NR_179901
HP1L	*Staphylococcus epidermidis*	HP1B1 (2)	99.64%	NR_113957
HP1M	*Carnobacterium antarcticum*	HP1B2 (3)	99.82%	NR_179901
HP1N	*Carnobacterium antarcticum*	HP1B2 (3)	99.55%	NR_179901
HP1O	*Carnobacterium antarcticum*	HP1B2 (3)	99.64%	NR_179901
HP1P	*Carnobacterium antarcticum*	HP1B2 (3)	99.55%	NR_179901
HP1Q	*Carnobacterium antarcticum*	HP1B2 (3)	99.37%	NR_179901
HP1R	*Carnobacterium antarcticum*	HP1B2 (4)	99.55%	NR_179901
HP1S	*Carnobacterium antarcticum*	HP1B2 (4)	99.82%	NR_179901
HP1T	*Carnobacterium antarcticum*	HP1B2 (4)	99.64%	NR_179901
HP1U	*Carnobacterium antarcticum*	HP1B2 (4)	99.03%	NR_179901
HP1V	*Carnobacterium antarcticum*	HP1B2 (4)	99.82%	NR_179901
HP2A	*Carnobacterium antarcticum*	HP2B1 (5)	99.29%	NR_179901
HP2B	*Carnobacterium antarcticum*	HP2B1 (5)	99.64%	NR_179901
HP2C	*Carnobacterium antarcticum*	HP2B1 (5)	99.82%	NR_179901
HP2D	*Carnobacterium antarcticum*	HP2B1 (5)	99.37%	NR_179901
HP2E	*Carnobacterium antarcticum*	HP2B1 (5)	99.03%	NR_179901
HP2F	*Carnobacterium antarcticum*	HP2B1 (6)	99.73%	NR_179901
HP2G	*Carnobacterium antarcticum*	HP2B1 (6)	99.91%	NR_179901
HP2H	*Carnobacterium antarcticum*	HP2B1 (6)	99.73%	NR_179901
HP2I	*Carnobacterium antarcticum*	HP2B1 (6)	99.73%	NR_179901
HP2L	*Carnobacterium antarcticum*	HP2B1 (6)	99.64%	NR_179901
HP2M	*Carnobacterium antarcticum*	HP2B2 (7)	99.55%	NR_179901
HP2N	*Staphylococcus epidermidis*	HP2B2 (7)	99.47%	NR_113957
HP2P	*Carnobacterium antarcticum*	HP2B2 (7)	99.46%	NR_179901
HP2Q	*Carnobacterium antarcticum*	HP2B2 (7)	99.82%	NR_179901
HP2R	*Carnobacterium antarcticum*	HP2B2 (8)	99.82%	NR_179901
HP2S	*Carnobacterium antarcticum*	HP2B2 (8)	99.91%	NR_179901
HP2T	*Carnobacterium antarcticum*	HP2B2 (8)	99.47%	NR_179901
HP2U	*Carnobacterium antarcticum*	HP2B2 (8)	99.73%	NR_179901
HP2V	*Carnobacterium antarcticum*	HP2B2 (8)	99.91%	NR_179901

HP1: Producer 1; HP2: Producer 2; B1: Batch 1; B2: Batch 2.

[a] Percentage of identical nucleotides between the sequence obtained from the lactic acid bacteria cultures and the sequence of the closest relative deposited in the GenBank database.

isolates in this specific food matrix is likely related to other metabolic activities rather than acid production. For this reason, the present study focused on evaluating their enzymatic activities instead of their acidifying capacity. It should be noted that the recovery of viable microorganisms through culture-dependent methods does not necessarily reflect their relative abundance in metataxonomic datasets, but rather their ability to survive, grow, and express functional traits under specific environmental and cultural conditions.

C. antarcticum is a Gram-positive, non-spore-forming, facultatively anaerobic bacterium characterized by short rod-shaped cells, typically 0.8–1.0 μm wide and 1.0–1.5 μm long, usually found singly, in pairs, or as short chains. This psychrotolerant and alkaliphilic species can grow over a temperature range of 4–36 °C, with an optimum between 28 and 32 °C. It tolerates pH values from 6.0 to 9.5, showing best growth at pH 8.0–8.5, and withstands NaCl concentrations up to 5% (w v^{-1}), with optimum growth around 1% (Zhu et al., 2018), thus explaining the occurrence of this species in the analyzed samples.

Members of the genus *Carnobacterium* have been isolated from varied environments, including the gastrointestinal tracts of fish and crustaceans, as well as from fermented fish products. In such settings, they play an important role in product preservation, safety, and flavor development (Ringø et al., 2002). Of particular interest are *C. maltaromaticum* and *C. divergens*, noted for their probiotic potential. These species produce bacteriocins and other antimicrobial agents that inhibit pathogens such as *Vibrio* and *Aeromonas* (Kim and Austin, 2008), making them promising candidates for use in aquaculture to improve fish health and disease resistance (Puvanendran et al., 2021).

However, some *Carnobacterium* species can act as opportunistic pathogens, especially in stressed hosts or cold-water environments. *C. maltaromaticum*, for example, has been associated with “pseudokidney disease” in salmonids, a condition involving chronic inflammation, pseudomembrane formation, and occasionally nephrocalcinosis, which can cause substantial mortality in fish farms and remains difficult to control (Loch et al., 2008).

The capacity of *Carnobacterium* to adapt to diverse substrates, particularly in fermented seafood, has been studied to better understand its influence on product quality and safety (Mauguin and Novel, 1994). In these products, the bacteria contribute to fermentation processes, inhibit spoilage, and enhance sensory attributes and shelf life, making them of technological interest to the food sector (Zotta et al., 2019).

The enzymatic activities assayed on the isolated cultures are reported in Fig. 5.

Among the isolates, only HP1R exhibited weak alkaline phosphatase activity. This trait is uncommon in carnobacteria; in fact, Ringø et al. (2002) reported no alkaline phosphatase reaction in numerous *Carnobacterium* strains, including *Carnobacterium divergens*, *Carnobacterium maltaromicus*, *Carnobacterium gallinarum*, *Carnobacterium mobile*, *Carnobacterium funditum*, *Carnobacterium alterfunditum*, and *Carnobacterium inhibens*.

Medium to high esterase (C4) activity was detected in half of the isolates, whereas only five showed medium esterase lipase (C8) activity. These results are consistent with those of Ringø et al. (2002), who

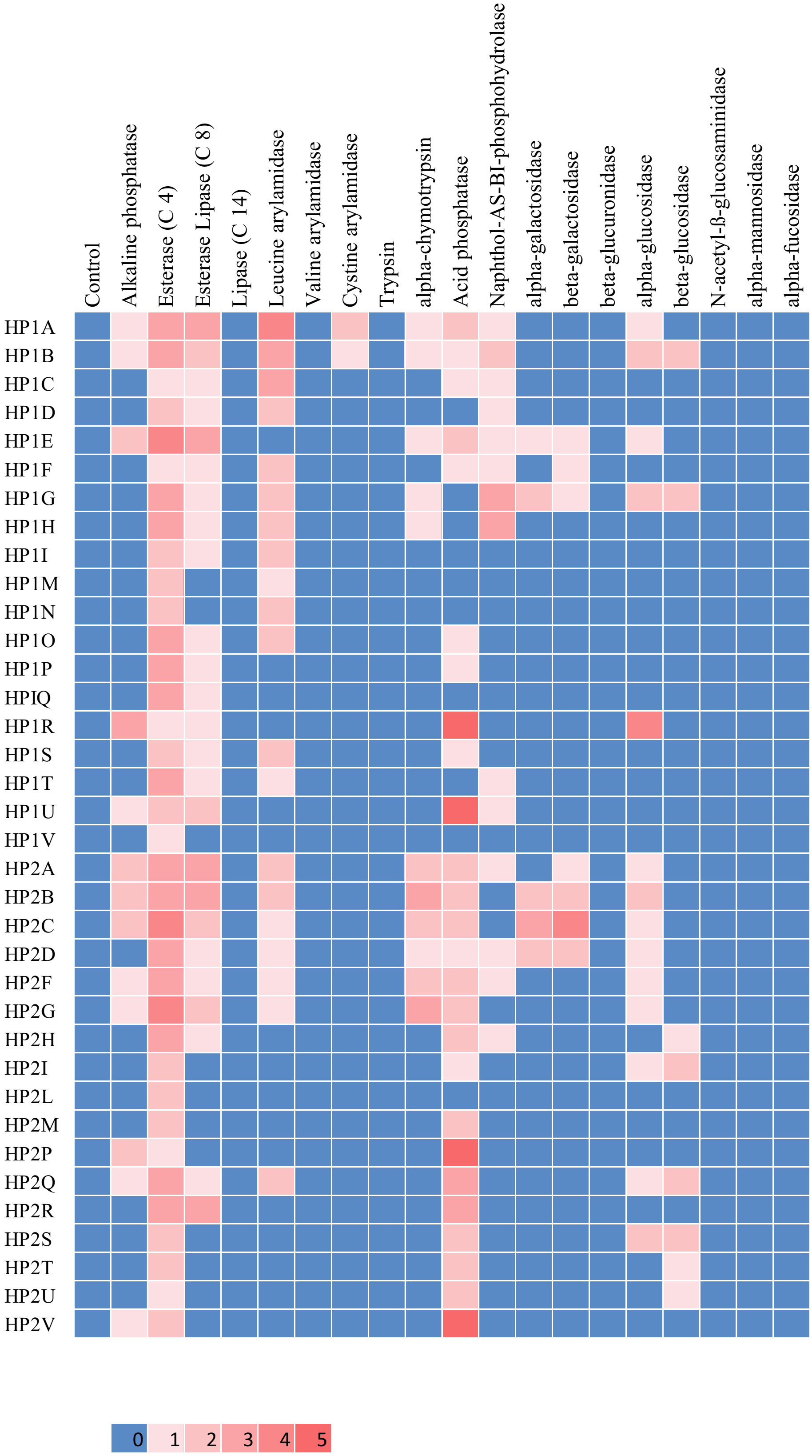

Fig. 5. Semi-quantitative analysis of the enzymatic activity exhibited by the lactic acid bacteria cultures isolated from *hákarl* samples. Each enzymatic reaction was scored on a scale from 0 to 5 based on the color intensity observed. A score of 0 indicated no reaction, represented by dark blue dots, whereas a score of 5 reflected the strongest reaction, shown as dark red dots. Scores of 1, 2, 3, or 4 represented intermediate levels of activity, with values of 3, 4, or 5 classified as positive reactions.

reported esterase activity in all the abovementioned tested *Carnobacterium* species except *C. inhibens*. Esterases comprise a broad and varied class of hydrolase enzymes that facilitate the breakdown or synthesis of ester linkages (Prim et al., 2006). Of note, the esterase activity exhibited by carnobacteria in the present study likely played a role in the formation of alcohols detected in the analyzed *hákarl* samples.

No lipase (C14) activity was detected in any isolate, supporting the observations of Ringø et al. (2002) for *Carnobacterium* strains.

Regarding leucine arylamidase, only three isolates (HP1A, HP1B, and HP1C) exhibited medium to high activity. In contrast, none displayed appreciable activity for valine arylamidase or cystine arylamidase. These findings align with those of Ringø et al. (2002), who reported no activity for these two enzymes in *Carnobacterium* strains. In fermented foods, these aminopeptidases play a crucial role in generating volatile compounds that shape the aromatic profile of the final product. Acting as exopeptidases, aminopeptidases specifically liberate amino acids located at the N-terminal of polypeptides and proteins (Gonzales and Robert-Baudouy, 1996). This mechanism allows them to break down proteins into oligopeptides and amino acids. Building on this capability, bacteria metabolize amino acids to generate key flavor compounds, such as aldehydes, alcohols, and esters (Cai et al., 2024). In the present study, leucine arylamidase activity detected in *Carnobacterium* may facilitate the release of branched-chain amino acids, which represent precursors for the formation of aldehydes and alcohols, whereas the release of aromatic amino acids may be involved in pathways leading to phenolic compounds, including phenol, detected in the *hákarl* volatilome.

Trypsin activity was absent in all isolates, in agreement with the results reported by Ringø et al. (2002) for *Carnobacterium*. A weak α-chymotrypsin activity was observed for two isolates (HP2B and HP2G). This enzyme is capable of performing proteolysis as well as ester hydrolysis (Flaschel and Ebmeier, 1998), thereby influencing the formation of volatile compounds.

Four isolates (HP1R, HP1U, HP2P, and HP2V) showed a strong acid phosphatase activity. This finding aligns with the observations of Ringø et al. (2002), who reported variable acid phosphatase activity among *Carnobacterium* strains. Phosphatases are enzymes that break down phosphoric acid esters through hydrolysis and are often referred to as phosphomonoesterases. Depending on their optimal pH, they are classified as either alkaline or acid phosphatases. Acid phosphatases function best at around pH 4.0 and are notably resistant to heat (Shaikh et al., 2023).

Only two isolates (HP1G and HP1H) exhibited weak naphthol-AS-BI-phosphohydrolase activity. To date, this enzymatic activity, responsible for the hydrolysis of phosphate esters, has been scarcely investigated in *Carnobacterium* and remains largely uncharacterized in lactic acid bacteria in general, limiting opportunities for comparison with existing data.

The isolate HP2C exhibited low α-galactosidase activity, an exoglycosidase that acts on galactooligosaccharides like raffinose, melibiose, stachyose, and certain branched polysaccharides (Bhatia et al., 2020), whereas the other isolates showed little to no detectable activity. Overall, these results are consistent with the enzymatic characterization reported by Ringø et al. (2002), who found no α-galactosidase activity in selected *Carnobacterium* strains. The same isolate showed a high β-galactosidase activity, whereas the remaining isolates displayed minimal or undetectable activity. Notably, β-galactosidase, also referred to as lactase, is a key enzyme of dairy lactic acid bacteria that breaks the β-glycosidic linkage connecting galactose and glucose (Kolev et al., 2022).

No isolate exhibited β-glucuronidase activity. This enzyme, a member of the glycosidase family, catalyzes the cleavage of O-glycosides to release β-D-glucuronic acid. The absence of such activity is considered advantageous, as bacteria expressing β-glucuronidase can contribute to the formation of carcinogenic compounds in the intestine (Lee et al., 2021).

Among the analyzed isolates, only HP1R exhibited strong α-glucosidase activity, an enzyme responsible for breaking down complex carbohydrates into simpler units.

None of the isolates exhibited appreciable activity for β-glucosidase (only weak activity was detected), *N*-acetyl-β-glucosaminidase, α-mannosidase, or α-fucosidase. These findings are consistent with the results reported by Ringø et al. (2002) and will not be further discussed.

3.6. Volatilome profile

Volatile organic compounds detected in the analyzed *hákarl* samples are reported in Table 6. Of note, unlike acid-fermented fish products, alkaline-fermented foods such as *hákarl* are typically characterized by a volatilome dominated by a limited number of high-impact compounds, mainly amines, sulfur-containing compounds, and phenols (Jang et al., 2017), rather than by a broad and complex spectrum of volatile metabolites. In the analyzed samples, 13 VOCs were identified including alcohols, aldehydes, ketones, phenols, sulfur- and nitrogen-containing compounds, whereas 5 VOCs remained unknown. Trimethylamine (TMA) was the predominant compound in samples of both producers, accounting for more than 50% of total volatiles. TMA is produced from trimethylamine oxide (TMAO), a natural compound present in fish capable of regulating hydrostatic pressures (Baliño-Zuazo and Barranco, 2016). TMA has a pungent fishery odor and, together with other nitrogen-containing compounds such as indole, has been found in other fermented fish products (Shen et al., 2021). Similarly, sulfur-containing compounds, specifically dimethyl sulfide and dimethyl trisulfide, are off-flavors with a very low odor threshold (Hu et al., 2025). Their presence was limited in the samples from the second producer (HP2), dimethyl trisulfide not being detected at all. Phenol was the second most abundant compound in samples of both producers (8–9%). It has a smoky and toasted flavor, and it was found in other fermented fish products, such as sea bass and mandarin fish, due to microbial degradation of tyrosine (Saito et al., 2018; Nie et al., 2022; Hu et al., 2025). Alcohols, aldehydes, and ketones were detected in both samples with no statistical differences, except for heptanal which was not detected in

Table 6
List of volatile organic compounds detected in *hákarl* samples.

Volatile organic compound	RI (est)	RI (lit)	HP1	HP2
Alcohols				
2-pentanol	1133	1114	0.58 ± 0.26	0.44 ± 0.21
Linolenic alcohol	1254	nr	1.51 ± 0.59	1.51 ± 0.40
3,4-dimethylpentanol	1357	1412	0.28 ± 0.08	0.81 ± 1.15
Phenolic				
oxime,- methoxy-phenyl-	1788		3.00 ± 2.31	1.61 ± 0.05
Phenol	2008	2008	7.97 ± 3.52	9.16 ± 0.70
Aldehydes				
pentanal, 2-methyl	445	nr	7.95 ± 1.20	5.98 ± 3.06
Heptanal	1213	1202	0.44 ± 0.01	nd
Ketones				
3-Pentanone	926	975	3.03 ± 0.65	5.95 ± 1.53
Sulfur-containing compounds				
Dimethyl disulfide	1063	1061	2.18 ± 1.42	0.54 ± 0.68
Dimethyl trisulfide	1364	1378	0.38 ± 0.54	nd
Nitrogen-containing compounds				
Alanine	399	nr	6.71 ± 1.49	5.76 ± 0.13
Trimethylamine	537	546	54.71 ± 8.01	57.97 ± 10.37
Indole	2470	2448	0.56 ± 0.44	0.47 ± 0.13
Others				
Unknown_1	491		3.15 ± 2.50	2.78 ± 1.02
Unknown_2	479		0.80 ± 0.21	0.94 ± 0.87
Unknown_3	902		3.84 ± 1.43	3.57 ± 3.67
Unknown_4	906		2.20 ± 1.10	2.10 ± 1.94
Unknown_5	1168		0.72 ± 0.17	0.41 ± 0.15

nr: no appropriate retention index reported in Nist database; nd: not detected.

HP2. These compounds are produced from the degradation and oxidation of lipids and amino acids, and they are associated with fermented fish smell (Shen et al., 2021; Ismaiel et al., 2025).

4. Conclusions

This study offers new insights into the microbial and physico-chemical features of *hákarl*, the traditional Icelandic fermented shark. By combining culture-dependent and culture-independent approaches, it provides a more comprehensive understanding of the product's microbiota. The analyses revealed the dominance of key taxa such as *T. creatinini* and *A. suicloacalis*, alongside the successful recovery of viable *C. antarcticum* isolates. Enzymatic characterization of these isolates demonstrated activities with potential technological relevance, particularly in flavor development, supporting their prospective use as functional cultures in food fermentation.

Physico-chemical analyses confirmed the strongly alkaline nature of *hákarl* and its lipid profile, typical of marine-derived products. In parallel, volatile profiling highlighted the predominance of TMA and phenolic compounds, which together shape the distinctive sensory attributes of the product. Notably, marked variability between producers was observed, underscoring the artisanal, non-standardized processing practices and their impact on microbial ecology, fatty acid composition, and volatile profiles.

Importantly, metagenomic analysis and culture-based isolation proved to be complementary, as the former may be influenced by DNA competition during extraction and amplification, while the latter depends on the viability of microorganisms at the time of sampling. Together, these methods provided a broader and more reliable picture of the microbial communities involved.

Overall, *hákarl* emerges as a culturally significant food and a valuable reservoir of microorganisms adapted to extreme conditions, with promising applications in food biotechnology. The results of the present study extend current knowledge, refine earlier observations, and establish a basis for future research aimed at valorizing *hákarl* as a model system for studying alkaline fermentations and microbial adaptation in traditional seafood.

Despite these findings, several limitations should be acknowledged. First, the study was conducted on commercially available ready-to-eat products, and detailed information regarding fermentation and drying conditions was not accessible, limiting the ability to directly link observed differences to specific processing parameters. Second, interpretations related to lipid oxidation and PUFA variability were not supported by direct measurements of oxidation markers (e.g., peroxide value or thiobarbituric acid reactive substances). In addition, the snapshot nature of the analysis does not allow assessment of microbial succession or biochemical dynamics throughout the fermentation process.

Future research should therefore focus on controlled, process-oriented studies to monitor microbial, chemical, and sensory changes during *hákarl* fermentation and drying, integrating oxidation indices, metabolomic approaches, and sensory analysis.

CRediT authorship contribution statement

Federica Cardinali: Writing – original draft, Investigation, Formal analysis. **Giorgia Rampanti:** Investigation, Formal analysis. **Paolo Lucci:** Resources, Investigation, Formal analysis. **Ilario Ferrocino:** Resources, Investigation, Formal analysis. **Deborah Pacetti:** Resources, Investigation, Formal analysis. **Benedetta Fanesi:** Investigation, Formal analysis. **Lama Ismaiel:** Investigation, Formal analysis. **Vesna Milanović:** Resources, Investigation, Formal analysis. **Cristiana Garofalo:** Resources, Formal analysis. **Annalisa Petruzzelli:** Investigation, Formal analysis. **David Savelli:** Investigation, Formal analysis. **Claudia Gabucci:** Investigation, Formal analysis. **Lucia Aquilanti:** Writing – review & editing, Resources. **Andrea Osimani:** Writing – review & editing, Supervision, Resources, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijfoodmicro.2026.111629.

Data availability

Data will be made available on request.

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