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Bacterial composition associated with different traditions of salted and dried fish across countries

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ABSTRACT

Drying of fish to improve storage capabilities, often under hyperosmotic conditions, is a widespread and long-standing practice in many cultures throughout the world. Several drying practices are applied, and they often reflect a cultural influence. The purpose of drying is to preserve fish by lowering the availability of water to microorganisms. However, because drying of fish is globally widespread and drying procedures are very diverse, microbial communities occurring in the dried fish products might differ. In this study, 63 dried fish products, prepared from 8 different fish species, were collected from several parts of the world (Greenland, Iceland, Denmark, Norway, the Faroe Islands, Japan, and Bangladesh) and the microbiotas from these products were analysed and compared following amplification and sequencing of the V4-region of the 16S rRNA. Overall, the dominant bacterial taxa associated with the fish were the genera *Photobacterium*, *Psychrobacteria*, *Vibrio*, and *Pseudomonas*, but large differences occurred across samples with a strong influence of the country of origin (in particular samples from Bangladesh) and salinity of the fish products. Moreover, industrially processed filets were readily distinguishable from traditionally processed ones. In contrast, the fish species from which the filets were prepared appeared to have less effect. These results suggest that drying practices can have a strong effect on the microbiota of the resulting products. For several of the fish species tested, this constitutes the first report regarding the composition of the microbiota associated with the resulting fish products.

1. Introduction

Drying has been used for the preservation of foods by humans for thousands of years (Theodoropoulou, 2014). This process significantly increases the storage time due to a reduced water activity (a_w), which inhibits microorganisms that are sensitive to low a_w. Indeed, most food-degrading bacteria are inhibited at a_w below 0.85, although a limited number of bacterial species and some fungi can still grow at a_w 0.75 (Beuchat, 1983; Bogasan et al., 2015; Jaykus et al., 2009). The effect of this drying process can be further enhanced with the addition of salt that increases osmotic pressure on potential food-spoiling bacteria and further lowers a_w. Salting reduces the number and variety of species of microorganisms being present (Chitrakar et al., 2019), but salting may also select for halophile or halotolerant microorganisms, e.g., species within the *Vibrio* genus, to the extent they can grow at a low a_w (Oliver, 2011).

Methods for the preservation of fish show large geographical and cultural differences and varies with the fish species and type of cut being used: In northern Europe, drying has historically been more commonly applied to preserve fish than fermentation and as a result, more variants of dried fish exist today than fermented kinds (Skåra et al., 2015). The traditional method of drying fish is simply exposure to outdoors air, sometimes after salting (Bogasan et al., 2015; Skåra et al., 2015). Usually, fish are unshielded from the surrounding environment, which increases the microbial density and composition, as compared to indoor drying (Chitrakar et al., 2019).

In Greenland, *Mallotus villosus* (whole capelin) is dried in the summer during fishing season when the weather is warm and sunny, and this facilitates a quick drying process (Hauptmann et al., 2020). In contrast, filleted *Gadus morhua* (cod) is traditionally dried during winter in open

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sheds. Cold and dry winter weather is ideal for drying cod due to their larger size, but requires a longer drying time, and the presence of only few flying insects. In Iceland, the traditional method for drying fish is similar to the approach used in Greenland, but sometimes the fish are washed in salt brine before drying (Hafstad, 2019). Due to hardening of the dried flesh, dried fish are locally named 'hardfish' or harðfiskur. In Norway, "stockfish" is produced from gutted cod that is dried outdoor on wooden racks in the winter (Bjørkevoll et al., 2003). After three months, the fish are brought indoor to mature further for up to 12 months. In the Faroe Islands, several species of lean fish are dried. Because of the slow gradual drying process, fermentation starts to occur in the fish tissues as bacteria spread through the filets. This is a tightly controlled process, and the fermentation and bacterial activity are interrupted by moving the filets to a drier environment if the fermentation proceeds too quickly (Svanberg, 2015). In Denmark, the only type of dried fish is *Limanda limanda* (flatfish dab) that is processed at coastal areas along the North Sea where strong winds are frequent (Green, 2015). The fish are treated in 10% salt brine for 24 h before washing in freshwater and drying for one to two weeks.

Drying of fish is not restricted to cold and temperate climates. For example, in the temperate regions of Japan, small sardines (named Niboshi) are boiled in salted water and dried before being used as flavour ingredients in many dishes (Adachi & Nozaki, 2001). In tropical climates, e.g., in central African countries, fish have traditionally been dried under the sun, but newer techniques, such as electric heating, vacuum drying and microwave treatment, have been introduced to reduce the drying time and ensure stable drying conditions and a low humidity (reviewed by Adeyeye, 2019). Sometimes, drying of fish is applied after fermentation to prevent further changes in taste, smell, texture, appearance, and nutritional value (Mohamed, 2015, pp. 161–182). In Bangladesh, sun-drying of fish in open air has been practised for centuries in the coastal regions (Alam, 2004; Balachandran, 2001). Unfortunately, the weather is changeable and does not always provide optimum conditions for drying, and this may, in combination with lack of proper hygiene, use of harmful chemicals, and improper processing, packaging and storage conditions, lead to a reduction in the quality of the filets, including microbial contamination and infestations with blow flies and beetles, lowering the market values of the fish (Alam, 2004; Begum et al., 2017; Hoque et al., 2021; Majumdar et al., 2017).

The aim of the present study was to describe the bacterial composition of dried fish products ready for consumption collected from several parts of the world (Greenland, Iceland, Denmark, Norway, the Faroe Islands, Japan, and Bangladesh) and identify the effect of various drying practices on the microbial composition in these products. We expected that the country of origin would affect the microbial composition due to cultural differences in the species being used and preparation practices. Differences in the drying process and salting were expected to be the dominant factors affecting composition. Because drying aims at reducing microbiological activity as much as possible, it was hypothesized that the drying process would strongly alter the original microbial composition of the microbiota in the fresh fish, affecting both richness and species composition, as identified using 16S rRNA sequencing of dried fish products across countries. Moreover, it was assumed that only a small number of euryhaline bacterial species could survive in salted fish, resulting in all samples having comparable microbiotas, and that the microbial assemblages would be dominated by the same few genera capable to survive in the salty environments and essentially removing the original diversity of the microbiota in the original unpreserved fish.

2. Material and methods

2.1. Samples

The fish samples used in this study were either industrially produced or obtained from private households (Table 1). From Greenland, three different types of dried cod were analysed: Industrially dried cod, purchased in frozen condition (www.royalgreenland.com); half-dried cod and winter-dried cod, produced in outdoor environments, but protected by roof and insect nets. Greenlandic capelins (ammassaat) were procured from a private household and had been kept at room temperature. The Norwegian traditionally dried Lofoten cod had been dried outdoors on wooden racks without head and guts and were afterwards kept at room temperature. From the Faroe Islands, the industrially produced fermented and dried haddock (*Melanogrammus aeglefinus*), cod and saithe (*Pollachius virens*) were kept at room temperature and packed separately in plastic when purchased. All three types of fish were produced in indoor drying-cabinets after being slightly fermented. The haddocks had been skinned and filleted, whereas the saithes were gutted and beheaded before being chopped into large chunks and dried. For the industrially dried cods, only the heads could be collected, but whole dried cod filets with skin (dried outdoor by hanging from a porch) were obtained frozen from a private household and included in the analysis. From Denmark, dabs were acquired in frozen condition and packed together in the same plastic bag. These fish had been dipped in a salty brine after being beheaded and gutted, after which they were dried outdoor on high-hanging racks. From Iceland, two brands of industrially produced cod from were packaged separately with several skinless filets in each plastic bag and were delivered at room temperature. The cods had both been salted before drying in indoor drying-chambers. The traditionally produced dried fish from Bangladesh, *Harpodon nehereus* (Bombay-duck) and *Trichiurus haumela* (ribbonfish), were transported to the laboratory at room temperature, with each fish packed separately in plastic bags. Finally, the industrially produced Japanese sardines were obtained packed together in plastic bags and had been stored at room temperature.

Table 1
Overview of the 63 samples in the dataset which were used in the analysis, showing number of samples, type of dried fish with the sample name in brackets, method of production, cut of fish, and the average salinity in.

Country	# samples	Type of dried fish	Method of production	Cut	Avg. Salinity (‰)
Bangladesh	9	Ribbonfish	Traditional	Gutted & beheaded	2.0
	9	Bombay Duck	Traditional	Gutted & beheaded	2.0
Denmark	4	Dab	Traditional	Gutted & beheaded	2.5
Faroe Islands	2	Cod	Traditional	Filet w. skin	1.3
	4	Haddock	Industrial	Filet	1.2
	1	Cod (Raesturcod)	Traditional	Gutted & beheaded	0.7
	8	Saithe (chops)	Industrial	Gutted & beheaded	0.7
	5	Cod	Industrial	Head only	0.7
Greenland	2	Cod (Half dried)	Industrial	Filet	1.2
	2	Cod (Machine dried)	Industrial	Filet	1.0
	2	Cod (Winter dried)	Industrial	Filet	0.7
	4	Capelin (Ammassat)	Traditional	mole	0.7
Iceland	3	Cod	Industrial	Filet	1.2
	2	Cod	Industrial	Filet	1.2
Japan	4	Sardine	Industrial	Whole	1.0
Norway	2	Cod (Lofoten)	Traditional	Gutted & beheaded	0.5

Each whole fish, chunk, head, or fillet were treated as an individual sample, regardless of having been stored in the same bag. After receiving each type of fish, they were stored at −20 °C before homogenization in a blender. After homogenizing each fish sample, the blender was cleaned

with ethanol and sprayed with RNase AWAY™ Surface Decontaminant (Thermo Fisher Scientific, Waltham, USA) to eliminate RNase and DNA contamination between the different fish samples.

2.2. Salinity

The salinity (ppt) of each fish sample was measured with a WTW Multi 3430 Meter (WTW, Weilheim, Germany), after adding 40 mL distilled water to 1 g sample, followed by thorough homogenization using a glass tissue grinder (Thomas Scientific, Swedesboro, USA).

2.3. DNA extraction and 16S rRNA gene amplicon sequencing

The extraction of DNA from the samples was done using 'MP Biomedicals FastDNA™ SPIN kit for soil' (MP Biomedicals, Irvine, USA) following standard protocol (Karst et al., 2016). In short, the samples were homogenized, and the proteins content was precipitated, then the DNA was bound to a silica matrix, after which the DNA was purified further, and lastly the DNA was eluted with DNase-free water. The obtained DNA was stored at −20 °C until preparation for sequencing.

The concentration of DNA was checked using the Qubit™ dsDNA BR/HS assay kit (Thermo Fisher Scientific, Waltham, USA) and for quality control of the DNA, the Tapestation 2200 with D1000 Screentape (Agilent, Santa Clara, USA) was used. Sequencing of the DNA was carried out with Nextera Amplicon Sequencing on an Illumina MiSeq platform, targeting the V4-region of the 16S rRNA as detailed elsewhere (Karst et al., 2016). Firstly, sample DNA was diluted accordingly, then the amplicon PCR was carried out in duplicates, pooling the samples afterwards to reduce the impact of PCR drift. After library clean-up using Agencourt AMPure XP (Beckman Coulter, Brea, CA, USA), quality control was carried out, and the libraries were pooled with a concentration of ~40 ng DNA/sample.

2.4. Data analysis

The raw reads of sequences were pre-processed with AmpProc5.1 (Yashiro, 2020), after which they were clustered into amplicon sequence variants (ASV) utilizing UNOISE3, which also removes chimeras and reads with sequencing errors (Edgar, 2016). The database SILVA (release S132) was used for genus identification, with a 99% level of similarity (Quast et al., 2013). Analysis of obtained data was performed using R version 3.6.3. (R Core Team, 2017), with package 'Ampvis 2' (Andersen et al., 2018).

Biodiversity was explored using alpha diversity, Chao1 (Chao, 1984). Because the data did not meet the assumptions of equal variances (as assessed using a Shapiro test), locations were therefore compared using a nonparametric Kruskal-Wallis test to assess differences between the medians of the distribution and pairwise comparisons using Dunns tests (Dunn, 1964). The microbial community structure was visualized using heatmaps of the 20 most abundant genera. To visualize the β-diversity, we used Correspondence Analysis (CA) on the Pearson's chi-squared (X^2) distance between each sample and the bacterial ASV therein and Permanova testing was performed to determine the effect of the various numeric and non-numeric factors (Buttigieg & Ramette, 2014). Prior to that, the ASV were transformed using a Hellinger transformation. The constrained version of CA, the Canonical Correspondence Analysis (CCA), was carried out to investigate whether country of origin or salt-content in the fish could explain the resulting groupings seen on the CA-plot.

3. Results

3.1. Diversity of bacterial communities

Sequencing of the amplicons corresponding to the V4 region of 16S rRNA gene yielded a total of 10,471 ASV. In total, 86% of the resulting sequences could be confidently identified at the family level while only 64% could be identified at the genus level. The resulting rarefaction curve showed that the curves flattened out after around 20,000 reads (Fig. S1). The rank-abundance curve (Fig. S2) shows that the highest count of rare ASV in the samples from the Faroe Islands, closely followed by the samples from Bangladesh, Japan and Greenland.

The number of observed ASV in the samples had high resemblance with the estimated chao1 richness (Fig. 1). The calculated chao1 values were significantly different between the Greenlandic 'ammassat' and two of the Faroese types of fish (cod head and saithe cutlets), and the Japanese sardine (p-value <0.05). Both the Japanese and the two Faroese fish species had the highest estimated richness (~4000 ASV). In contrast, the ammassat from Greenland had the lowest estimated richness with a mean chao1 value of 1172 (Fig. 1).

Differences in the community structure between samples (β-diversity) were visualized by correspondence analysis (CA) and showed that multiple components contributed to the total variance between samples (Fig. 2) and that the CA plot explained a total of 21.1% of the correspondence in the data. No single factor made a major contribution to the diversity, as shown on the Scree plot (Fig. S3). However, the most influential factors were the country of origin of the samples ($R^2 = 0.57$, $p = 0.001$) and the average salinity of the filets ($R^2 = 0.32$, $p = 0.001$). The processing method had a more limited, but still detectable influence on the β-diversity ($R^2 = 0.2179$, $p = 0.001$).

To further explore the parameters associated with differences in the microbiotas across samples and increase resolution in the other samples and factors shaping these differences, we investigated the β-diversity using country of origin and salinity in the CCA (Fig. S4), but excluded the samples from Japan and Bangladesh as these samples were very different and this could have masked smaller effects. The axes show two percentages; on the left is the total inertia of the correspondence, and on the right the percentage-wise inertia of the chosen constraint is shown. When using country of origin as the constrain, three major groupings were evident, namely samples from Greenland, Iceland, and the remaining samples (Fig. S4A). Using salinity as the constrain, a further grouping is noted, where samples from Denmark and Iceland are also grouping individually (Fig. S4B).

3.2. Bacterial community composition

The bacterial taxa associated with the fish samples showed that the microbiota were dominated by the genera *Photobacterium, Psychrobacteria, Vibrio,* and *Pseudomonas*, but large differences could be observed between samples (Fig. 3). In particular, the geographic origin of the samples had a clear effect, as expected from the CCA analyses shown above. Thus, the samples from Europe and the North Atlantic showed somewhat similar pattern, except for half of the samples from Iceland (Codfilet B) and the samples from Greenland that were deviating from the other samples. In most of the European and North Atlantic samples, members of the genus *Photobacterium* were dominant, followed by members of the genus *Psychrophilum.* A similar pattern was observed among samples of Codfilet A from Iceland. In contrast, samples from Codfilet B from Iceland were unique and dominated by *Kocuria* spp. and *Carnobaterium* spp. Samples from Greenland showed more variability, with the ammassat samples being more comparable to other samples from the region and dominated by vibrios and with *Photobacterium* and *Psychrobacteria* being the other main groups. Like the European and North Atlantic samples, the Japanese sardines were dominated by *Photobacterium* and members of the *Vibrio* genus. Finally, samples from Bangladesh were the most different as compared to samples from other countries and were dominated by *Psychrobacter* spp. and *Pseudomonas* spp.

Comparison of the composition of microbiotas from fish at different salinity showed a progressive reduction in the abundance of some species. Notably, *Pseudomonas*, *Shewanella*, and *Proteus* sp. were fairly common at salinities below 1.6 ppt, but were reduced in abundance at

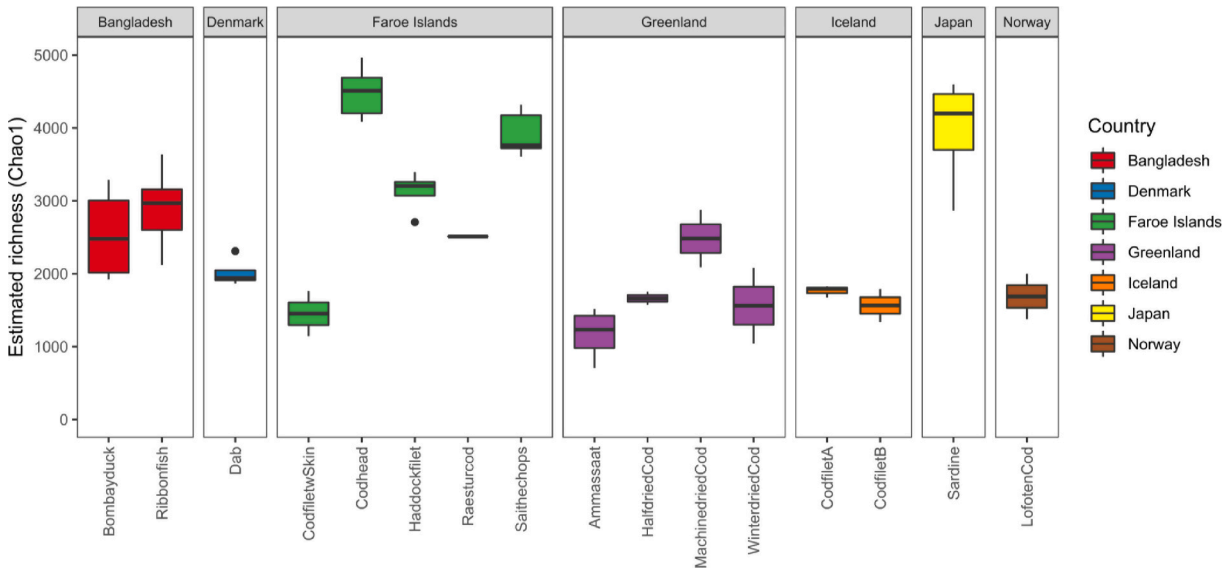

Fig. 1. Boxplot displaying the richness (Chao1) of samples sorted by type of fish and country of origin. The boxplot bounds the interquartile range (IQR) divided by the median; the whiskers extend to 1.5x IQR beyond the box. Dots indicate outliers (N = 1–9).

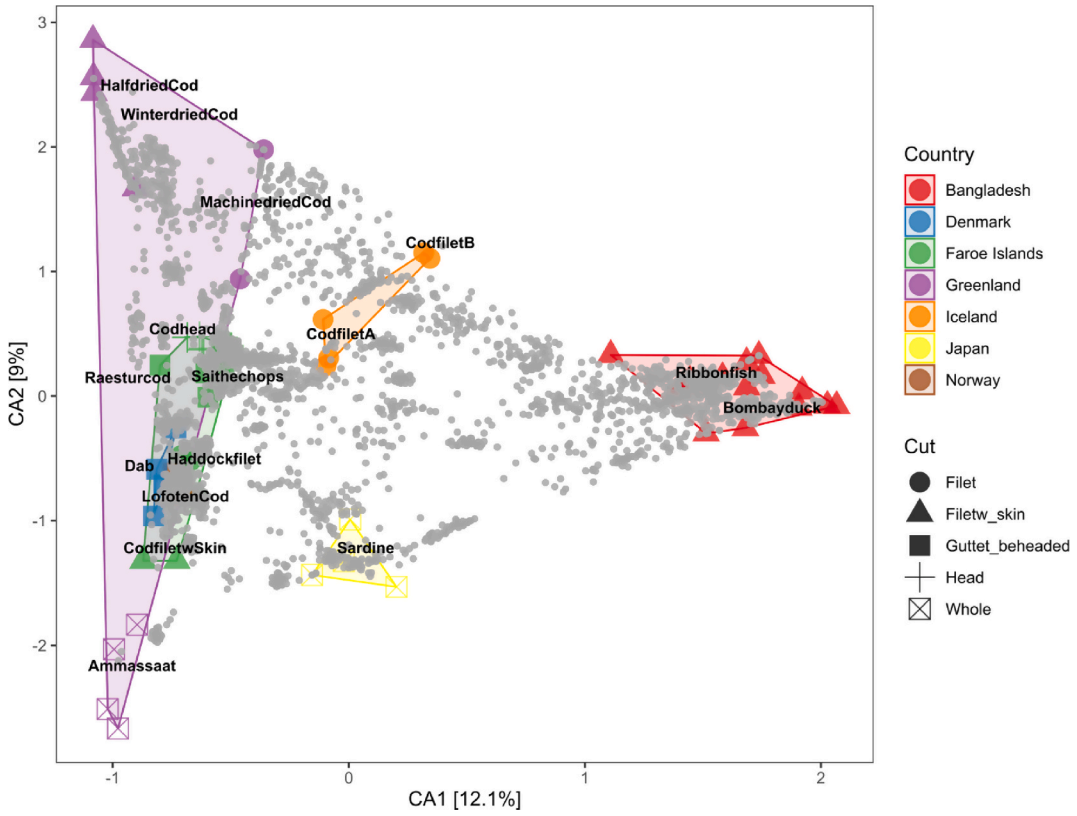

Fig. 2. Correspondence analysis of the samples, with the sample description written on the plot. The colours indicate country of origin of samples, and the shape of the point refers to the cut of fish. This plot explains a total of 21.1% of the correspondence between the samples. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

higher salinities. Other genera, such as *Psychrilyobacter*, *Fusobacterium*, or *Lysinibacillus* sp., were only present at salinities below 1.6 ppt and absent at higher salinities. In contrast, the dominant genera such as *Photobacterium*, *Psychrobacter*, and *Vibrio* sp. were mostly unaffected by the changes in salinity.

Despite its limited effect on β-diversity, the industrial processing methods still influenced the composition of the microbiota. Samples of the machine-processed cods were dominated by members of *Pseudomonas* and *Shewanella*, while the half-dried cod and the winter dried cod were overwhelmingly dominated by representants of the genus *Psychrobacter* (over 92% of the identified sequences in the case of half-dried cod) (Fig. S5). At the Faroe Islands, traditional methods were associated with a reduction of the diversity alongside a strong increase in the abundance of *Moritella* spp. In Greenland, members of the genus *Vibrio*

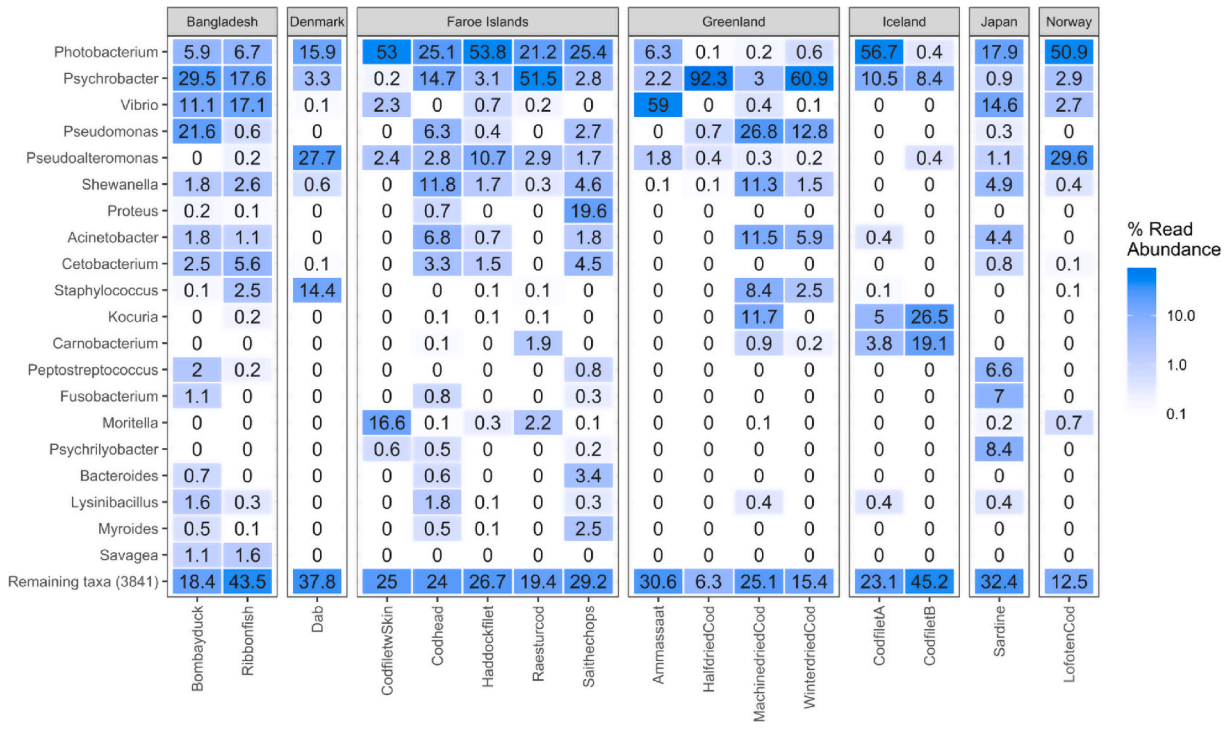

Fig. 3. Heatmap (mean) showing the 20 most abundant genera of bacteria of samples. Samples are sorted by type of fish sample and country of origin. Shade of colours correspond to the percentagewise read abundance. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

had a greater abundance in fish produced by the traditional method, as compared to *Psychrobacter* sp. that were reduced in abundance.

4. Discussion

In the present study, we found a strong effect of specific drying practices on the composition of the microbiotas using 16S rRNA amplicon sequencing. Effects of the drying practises were illustrated by a strong impact of the country of origin of the samples, but also of the salinity of the products. This contrasts the results reported by Hauptmann and co-authors for dried cod in Greenland, as they observed that the microbiota were not affected by different drying methods, including salting, no salting, or saltwater (Hauptmann et al., 2020). Further, we found that the microbiota differed between traditionally and industrially processed cod. This is also in contrast to the results of Hauptmann and coauthors, who only found such an effect for capelin but not for cod (Hauptmann et al., 2020). In their study, the authors performed analysis based on OTU rather than ASV, which may explain some differences between the two studies as ASV are generally considered more sensitive at picking up small differences in bacterial richness.

The microbiota in the samples from Bangladesh was dominated by *Psychrobacter* spp., *Vibrio* spp., and, in the case of Bombay-duck fish (*H. nehereus), Pseudomonas* spp. Meanwhile, *Staphylococcus* spp. was only detected at low level, and neither *E. coli*, *Bacillus* spp. nor *Salmonella* spp. were among the 20 most abundant genera. These findings contrast the observation by Hassan et al. (2021) who reported that the most commonly isolated bacterial species in dried fish sold in Bangladesh are *Staphylococcus* spp. and *Bacillus* spp. Similarly, sampling of dried *Mastacembelus armatus* fish from producers and retail markets in Bangladesh showed that *E. coli* were found on all fish, and most were also contaminated with *Salmonella* spp. (Akter et al., 2018). The reason for these discrepancies is not known, but several explanations exist: The authors investigated how commonly these species could be isolated from the samples, while our investigation focused on evaluating the number of bacterial taxa in each sample. It is possible that *E. coli*, *Bacillus* spp. and *Salmonella* spp. were present in all tested samples but were overlooked in our study due to a low abundance. However, it seems somewhat unlikely that such widespread bacteria would not be more numerous in the samples. Alternatively, these authors relied on cultivation techniques instead of sequencing techniques as we did, and cultivation techniques are well-known to result in significant bias when investigating microbiotas (Spanggaard et al., 2000). Moreover, the fish species likely had an impact on the microbiota. Thus, Akter et al. (2018) sampled *Mastacembelus armatus,* while Hassan et al. (2021) did not identify the fish species sampled.

Other studies on drying of fish for conservation have focused on different fish species. For example, Jääskeläinen et al. (2019) reported that *Photobacterium* spp. was the dominant species in *Salmo salar* (Atlantic salmon) packaged in Norway, while *Pseudomonas* spp. dominated samples prepared from *Thunnus albacares* (yellowfin tuna) from the Maldives. These results are comparable to ours, as *Photobacterium* spp. and *Pseudomonas* spp. were among the most common genera identified in the fish studied, with the notable exception of fish originating from Greenland. Interestingly, increased prevalence of *Pseudomonas* spp. has been reported in the microbiota of filets from freshwater *Hypophthalmichthys nobilis* (bighead carp) in association with the salting process (Liu et al., 2017). Moreover, the importance of this genus has been reported to increase over time (Zhang et al., 2021) and might be an indicator of spoilage in stored fish filets (Wang & Xie, 2020).

Although we did not examine microbiota of the fresh fish, reports on microorganisms in fresh fish indicate that the composition of the microbiota between dried and fresh fish differs significantly. Thus, previous studies of microbes on living and freshly caught marine fish showed that various Gram-negative species are common, e.g., *Pseudomonas*, *Acinetobacter*, *Shewanella* and *Flavobacterium*, while Gram-positive species, e.g., *Bacillus*, *Micrococcus*, and *Clostridium*, are less abundant (reviewed in Leisner & Gram, 2000). In processed fish, other microbiotas are often more abundant. For example, psychrophilic species like *Pseudomonas* sp. and *Shewanella* sp. are common in cold-stored fish, while the halophilic species *Halobacterium* and *Halococcus* may

occur in salted fish (Leisner & Gram, 2000). In our samples, the most abundant genera were *Photobacterium* sp., *Phychrobacter* sp., *Pseudomonas* sp., and to a lesser extent *Vibrio* and *Shewanella* sp. All of these genera are known to be associated with the spoilage of fish products, including fresh products. This suggests that drying slows down spoilage by reducing the rate of bacterial activity, rather than outright eliminating the bacteria responsible for the spoilage.

For several fish species, the present study represents the first report on microbiota colonising the associated dried fish products. In addition, this study relies on sequencing of the 16S rRNA gene, while microbiological analyses of fish and seafood have until recently mainly been based upon classical cultivation-based methods, e.g., according to the standards of the International Organization for Standardization (ISO) for identification of psychrotrophic microorganisms (ISO17410:2019, 2019). However, cultivation-based approaches overlook species that do not grow on standard laboratory media or are present at low abundance. Moreover, even culturable microorganisms might not grow on standard media and might not be detectable without special corrections to standard protocols (Broekaert et al., 2011). In addition, culture methods are biased toward fast-growing organisms, meaning that slower-growing organisms can often be overlooked. Several phychrotrophic and potential fish-degrading microbes are also not detected by the ISO 17410 -2019 plating methods, but may be detected using molecular approaches. For example, in a recent study molecular approaches were applied for analysis of microbiotas on skin of salt-dried codfish, and more than 245 operational taxonomic units (OTUs), dominated by Gammaproteobacteria, such as *Pseudomonas*, *Serratia*, *Salinisphaera* and *Psychrobacter*, were identified (Pegoraro et al., 2015). This diversity would not have been revealed by plating methods, indicating that sequencing approaches are valuable tools for analysis of microbiomes on dried fish.

Conversely, one limitation of DNA analysis is that it does not differentiate between living and dead organisms. It is therefore plausible that a number of organisms detected in the present study were no longer viable (Young et al., 2007). However, the fact that the microbiotas were clearly different from those associated with living fish, suggests that this effect was limited and that most of the microbiota identified originated from colonisation of the fish after the salting treatment.

5. Conclusions

In the present study, the effect of different drying methods was compared for various samples of dried fish. Overall, country of origin of the samples were found to have the strongest effect on the microbiotas on the products, but drying methods (salinity and traditional vs. industrial) also affected the composition, suggesting a significant impact of cultural practices on the final products. Drying is a widespread method for the conservation of foodstuff and has a long-established tradition throughout the world. The present study represents the first report on the composition of microbiotas in dried products from several fish species and more particularly, application of molecular techniques for analysis of the microbiotas. This study will improve our understanding of how drying procedures impact the microbiota of fish products, and it provides a platform for future studies for comparison of similar observations of microbes in fish products.

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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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fbio.2022.101991.

References

Adachi, M., & Nozaki, Y. (2001). The effects of the storage temperature and the size of Niboshi on the quality of stored Niboshi. *Nippon Shokuhin Kagaku Kokagu Kaishi, 48* (3), 214–217. https://doi.org/10.3136/nskkk.48.214

Adeyeye, S. A. O. (2019). An overview of fish drying kinetics. *Nutrition and Food Science, 49*(5), 886–902. https://doi.org/10.1108/NFS-10-2018-0296

Akter, T., Sayeed, A., Rasul, G., Kashem, A., & Paul, A. K. (2018). Evaluation of microbiological quality of dried baim (*Mastacembelus armatus*) in Bangladesh. *Archives of Agriculture and Environmental Science, 3*(4), 344–353. https://doi.org/24566632.2018.030403.

Alam, N. (2004). Traditional fish drying in coastal villages of Bangladesh: Practices , constraints and scope for improvements. In *Low-cost fish processing in coastal Bangladesh* (pp. 1–45). FAO.

Andersen, K. S., Kirkegaard, R. H., Karst, S. M., & Albertsen, M. (2018). Ampvis2: an R package to analyse and visualise 16S rRNA amplicon data. *BioRxiv*. , Article 299537. https://doi.org/10.1101/299537

Balachandran, K. K. (2001). *Postharvest technology of fish and fish products*. Daya Publishing House.

Begum, A., Syed, A., & Alam, N. (2017). Detection, quantification and decontamination of pesticide residues in dry fish. *International Journal of Biosciences, 10*(6), 246–252.

Beuchat, L. R. (1983). Influence of water activity on growth, metabolic activities and survival of yeasts and molds. *Journal of Food Protection, 46*(2), 135–141. https://doi.org/10.4315/0362-028x-46.2.135

Bjørkevoll, I., Olsen, R. L., & Skjerdal, O. T. (2003). Origin and spoilage potential of the microbiota dominating genus *Psychrobacter* in sterile rehydrated salt-cured and dried salt-cured cod (*Gadus morhua*). *International Journal of Food Microbiology, 84*(2), 175–187.

Bogasan, C. S., Nero, L. A., & Todorov, S. D. (2015). From traditional knowdledge to an innovative approach for bio-preservation in food by using lactic acid bacteria. In M. T. Liong, & A. Steinbüchel (Eds.), *Beneficial microorganisms in food and nutraceuticals*. Springer.

Broekaert, K., Heyndrickx, M., Herman, L., Devlieghere, F., & Vlaemynck, G. (2011). Seafood quality analysis: Molecular identification of dominant microbiota after ice storage on several general growth media. *Food Microbiology, 28*(6), 1162–1169. https://doi.org/10.1016/j.fm.2011.03.009

Buttigieg, P. L., & Ramette, A. (2014). A guide to statistical analysis in microbial ecology: A community-focused, living review of multivariate data analyses. *FEMS Microbiology Ecology, 90*(3), 543–550. https://doi.org/10.1111/1574-6941.12437

Chao, A. (1984). Non-parametric estimation of the number of classes in a population. *Scandinavian Journal of Statistics, 11*, 265–270.

Chitrakar, B., Zhang, M., & Adhikari, B. (2019). Dehydrated foods: Are they microbiologically safe? *Critical Reviews in Food Science and Nutrition, 59*(17), 2734–2745. https://doi.org/10.1080/10408398.2018.1466265

Dunn, O. J. (1964). Multiple comparisons using rank sums. *Technometrics, 6*(3), 241–252.

Edgar, R. (2016). UNOISE2: Improved error-correction for Illumina 16S and ITS amplicon sequencing. *BioRxiv*. , Article 081257. https://doi.org/10.1101/081257

Green, A. (2015). Flounder and dab. In *Field guide to seafood* (p. 58). Quirk Books.

Hafstad, V. (2019). How dried fish is made. *Iceland Monitor*. https://icelandmonitor.mbl.is/news/eat_and_drink/2019/06/12/how_dried_fish_is_made/.

Hassan, M. K., Poppy, M., Ahamed, T., Morsheda, F., Yadav, R., Hasan, A., & Siddique, M. P. (2021). Bacterial flora isolated from dried fishes sold at retail markets within Dhaka city corporation of Bangladesh. *Research in Agriculture Livestock and Fisheries, 8*(1), 109–115. https://doi.org/10.3329/ralf.v8i1.53272

Hauptmann, A. L., Paulová, P., Castro-Mejía, J. L., Hansen, L. H., Sicheritz-Pontén, T., Mulvad, G., & Nielsen, D. S. (2020). The microbial composition of dried fish prepared according to Greenlandic Inuit traditions and industrial counterparts. *Food Microbiology, 85*, Article 103305. https://doi.org/10.1016/j.fm.2019.103305

Hoque, M. S., Tamanna, F., Hasan, M. M., Al Banna, M. H., Mondal, P., Prodhan, M. D. H., Rahman, M. Z., & van Brakel, M. L. (2021). Probabilistic public health risks associated with pesticides and heavy metal exposure through consumption of common dried fish in coastal regions of Bangladesh. *Environmental Science and Pollution Research.* , Article 0123456789. https://doi.org/10.1007/s11356-021-17127-9

ISO17410:2019. (2019). *ISO 17410:2019. Microbiology of the food chain — horizontal method for the enumeration of psychrotrophic microorganisms*.

Jääskeläinen, E., Jakobsen, L. M. A., Hultman, J., Eggers, N., Bertram, H. C., & Björkroth, J. (2019). Metabolomics and bacterial diversity of packaged yellowfin tuna (*Thunnus albacares*) and salmon (*Salmo salar*) show fish species-specific spoilage development during chilled storage. August 2018 *International Journal of Food Microbiology, 293*, 44–52. https://doi.org/10.1016/j.ijfoodmicro.2018.12.021.

Jaykus, L. A., Wang, H. H., & Schlesinger, L. S. (2009). *Food-borne microbes: Shaping the host ecosystem.* ASM Press. ASM Press.

Karst, S. M., Albertsen, M., & Kirkegaard, R. H. (2016). Chapter 8 : Molecular methods for identification of microorganisms. In *Experimental methods in wastewater treatment* (pp. 1–17).

Leisner, J., & Gram, L. (2000). Spoilage of fish products. In R. Robinson, C. Batt, & P. Patel (Eds.), *Encyclopedia of food microbiology* (pp. 813–820). Academic Press.

Liu, X., Zhang, Y., Li, D., & Luo, Y. (2017). Characterization of the microbiota in lightly salted bighead carp (Aristichthys nobilis) fillets stored at 4 °C. *Food Microbiology, 62*, 106–111. https://doi.org/10.1016/j.fm.2016.10.007

Majumdar, B. C., Afrin, F., & Rasul, M. G. (2017). Comparative study of physical, chemical, microbiological and sensory aspects of some sun dried fishes in Bangladesh. *Brazilian Journal of Biological Sciences, 4*(8), 323–331.

Mohamed, G. A. E. H. (2015). *Fermented fish products in Sudan*. https://doi.org/10.1007/978-3-319-23177-8_7

Oliver, J. D. (2011). Chapter 18 Culture media for the isolation and enumeration of pathogenic Vibrio species in foods and environmental samples. In J. E. L. Corry, G. D. W. Curtis, & R. M. Baird (Eds.), *Handbook of culture media for food and water microbiology* (3rd ed., pp. 377–402). Royal Society of Chemistry. https://doi.org/10.1039/9781847551450-00377.

Pegoraro, N., Calado, R., Duarte, L. N., Manco, S. C., Fernandes, F. J., Polónia, A. R. M., Cleary, D. F. R., & Gomes, N. C. M. (2015). Molecular analysis of skin bacterial assemblages from codfish and pollock after dry-salted fish production. *Journal of Food Protection, 78*(5), 983–989. https://doi.org/10.4315/0362-028X.JFP-14-390

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., & Glöckner, F. O. (2013). The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. Database issue *Nucleic Acids Research, 41*, D590–D596. https://doi.org/10.1093/nar/gks1219.

R Core Team. (2017). *R: A language and environment for statistical computing*. R.

Skåra, T., Axelsson, L., Stefánsson, G., Ekstrand, B., & Hagen, H. (2015). Fermented and ripened fish products in the northern European countries. *Journal of Ethnic Foods, 2*(1), 18–24. https://doi.org/10.1016/j.jef.2015.02.004

Spanggaard, B., Huber, I., Nielsen, J. L., Nielsen, T., Appel, K. F., & Gram, L. (2000). The microflora of rainbow trout intestine: A comparison of traditional and molecular identification. *Aquaculture, 182*(1–2), 1–15. https://doi.org/10.1016/S0044-8486(99)00250-1

Svanberg, I. (2015). Ræstur fiskur: Air-dried fermented fish the Faroese way. *Journal of Ethnobiology and Ethnomedicine, 11*(1), 76. https://doi.org/10.1186/s13002-015-0064-9

Theodoropoulou, T. (2014). Salting the east: Evidence for salted fish and fish products from the aegean sea in roman times, 2014. In E. Botte, & V. Leitch (Eds.). *Fish & ships*. Publications du Centre Camille Jullian. https://doi.org/10.4000/books.pccj.1738. Issue January 2014.

Wang, X.-Y., & Xie, J. (2020). Growth kinetics and spoilage potential of Co-culturing *Acinetobacter johnsonii* and *Pseudomonas fluorescens* from bigeye tuna (*Thunnus obesus*) during refrigerated storage. *Current Microbiology, 77*(8), 1637–1646. https://doi.org/10.1007/s00284-020-01978-5

Yashiro, E. (2020). *AmpProc5 ASV pipeline*. https://github.com/eyashiro/AmpProc.

Young, G., Turner, S., Davies, J. K., Sundqvist, G., & Figdor, D. (2007). Bacterial DNA persists for extended periods after cell death. *Journal of Endodontics, 33*(12), 1417–1420. https://doi.org/10.1016/j.joen.2007.09.002

Zhang, Q., Chen, X., Ding, Y., Ke, Z., Zhou, X., & Zhang, J. (2021). Diversity and succession of the microbial community and its correlation with lipid oxidation in dry-cured black carp (Mylopharyngodon piceus) during storage. *Food Microbiology, 98*, Article 103686. https://doi.org/10.1016/j.fm.2020.103686