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ORIGINAL ARTICLE

Bacterial diversity of smoked and smoked-dried fish from West Africa: A metagenomic approach

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Abstract

This study aimed to explore the bacterial diversity of smoked fish and smoked-dried fish. Forty-eight fish samples were collected from various processing sites and markets in Benin. The bacterial diversity was analyzed using high-throughput sequencing of the 16S rRNA gene on the Illumina MiSeq platform. In total, 16 bacterial phyla were identified across all samples, with the majority of sequences belonging to Firmicutes (43.3%) and Proteobacteria (43.6%). Families, Staphylococcaceae, Moraxellaceae, Planococcaceae, Enterobacteriaceae, Vibrionaceae, and Bartonellaceae, were well represented. A total of 384 distinct genera was identified, with the most abundant represented by the Gram-negative, *Acinetobacter*, *Bartonella*, *Enterobacter*, *Morganella*, and *Photobacterium*, and the Gram-positive, *Aerococcus*, *Bacillus*, *Kurthia*, *Macrococcus*, *Staphylococcus*, and *Weissella*. OTUs related to pathogenic bacteria, such as *Salmonella* spp., *Staphylococcus aureus*, and *Listeria monocytogenes*, were not detected in these popular foods sold in street markets in Benin. However, the presence of potentially harmful histamine-producing bacteria has been revealed.

Practical application: In West Africa, fish is mainly preserved by hot smoking, which can be followed by an additional drying step using the heat that emanates from the kiln. Although several studies have investigated the microbiological quality of smoked and smoked-dried fish, their overall microbial diversity has remained so far poorly explored. Therefore, this study has investigated the bacterial diversity of these popular foods. The results of this work provide useful information on bacteria potentially participating in food spoilage or compromising the safety of these popular foods and can be useful for improving their quality and safety.

1 | INTRODUCTION

Fish, through its interesting nutritional composition, is an excellent complement to the Western African diet, which is essentially based on cereals and starchy foods (Adeyeye et al., 2015; Iko Afé et al., 2020; Ikutegbe & Sikoki, 2014). Smoked fish products are highly appreciated for their organoleptic properties (Aygül et al., 2010; Daramola et al., 2014) and are consumed by the vast majority of West African people. Fish is, however, highly perishable due to autolytic and microbial spoilage that occur just after death (Dehghani et al., 2018). Moreover, smoked fish products are extensively handled, by both processors and customers, which results in the development of unpleasant odors and compromises their sanitary conditions (Assogba et al., 2019). As popular food products, several studies have focused on their microbiological quality and safety using conventional microbiological methods (Adeyeye et al., 2015; Anihouvi et al., 2019; Ayeloja et al., 2018; Ineyougha et al., 2015). Most of these studies have however been limited to the detection of

common spoilage and pathogenic microorganisms and few of these reports explored the total viable microflora in these food products. The above researchers used culture-dependent methods and biochemical tests in their experiments, which cannot address all the bacterial diversity contained in food matrices.

With the advance of molecular methods, including 16S rRNA amplicon sequencing, it is now possible to better assess the extent of diversity of bacterial communities, with a much better resolution than culture-dependent methods (De Filippis et al., 2018; Jia et al., 2018; Kergourlay et al., 2015; Parlapani, 2021; Zotta et al., 2019). Using amplicon sequencing approach, the complexity of microbial communities associated with the aquatic environment (Fiore et al., 2019), food stuffs (Azi et al., 2019; Hu et al., 2020; Wang et al., 2017), fish (Jia et al., 2018; Kuuliala et al., 2018; Parlapani et al., 2018; Rosado et al., 2018; Zotta et al., 2019), and other seafood (Parlapani et al., 2020) have recently been approached.

The present study aimed to further explore the diversity of bacterial communities found in these foods using an amplicon sequencing approach. SF and SDF were collected from various processing plants and markets for investigating the composition and diversity of their associated bacterial community.

2 | MATERIALS AND METHODS

2.1 | Origin of investigated samples

A selection of 48 smoked and smoked-dried fish samples of six species was analyzed in this study (Table 1): Atlantic mackerel (*Scomber scombrus*), Benguela hake (*Merluccius polli*), Nile tilapia (*Oreochromis niloticus*), Flying fish (*Cypselurus cyanopterus*), Barracuda (*Sphyraena baraccuda*), and Bonga shad (*Ethmalosa fimbriata*). For each fish species, eight samples were randomly purchased from different processors in different municipalities of Benin. Samples were placed in sterile plastic bags and immediately transferred into a refrigerated cold box (about 4°C) to the laboratory where they were stored in freezers (−20°C).

2.2 | Total DNA extraction

DNA extraction was performed accordingly to Zotta et al. (2019) with some modifications. Briefly, for each sample, 5 g (skin and fillet) with a water content of 19%–67%, which covers as much or more biological material than the 10 g to 25 g of fresh fish or shrimp as used in previous studies (Parlapani et al., 2018, 2020; Zotta et al., 2019), were suspended in 45 ml of buffered peptone water (Bio-Rad, pH 7.0 ± 0.2), and homogenized (230 rpm, 2 min) in a stomacher (Lab Blender, Model 400, Seward Medical, London) to obtain a 1/10 dilution. DNA extraction was done directly from the 1/10 dilution. For this purpose, 1.8 ml of 1/10 dilution were centrifuged in an Eppendorf for 3 min at 13,000 × g and 4°C. The supernatant was removed and the tube was centrifuged for 3 min to remove the remainder of the supernatant. The pellet was used to extract the DNA using the NucleoSpin®Food (Macherey-Nagel GmbH & Co) following the manufacturer's recommendations. The resulting DNA extracts were eluted in ultrapure water and stored at −20°C until used for 16S rRNA amplicon sequencing.

2.3 | Bacterial 16S RRNA gene sequencing and bioinformatics analysis

High-throughput sequencing was performed on SF and SDF samples to assess their bacterial diversity and relative abundance. DNA concentration of each DNA extract was measured with a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE, USA) and normalized with ultrapure water to a final concentration of 20 ng/µl. Purified DNA samples were then processed and sequenced on Illumina MiSeq at RTL Genomics (Lubbock, TX, USA). The sequenced DNA covered the V1-V2 hypervariable region of the 16S rRNA gene. The forward primer was constructed with the Illumina i5 adapter, an 8–10 bp barcode and primer 28F (5′-GAGTTTGATCNTGGCTCAG −3′). The reverse primer was constructed with the Illumina i7 adapter, an 8–10 bp barcode and primer 388R (5′-TGCTGCCTCCCGTAGGAGT-3′).

The amplification mix was prepared with Qiagen HotStar Taq master mix, supplemented with 0.25 µM of each primer. The thermal profile of amplification was 95°C for 5 min, then 35 cycles of 94°C for 30 s, 54°C for 40 s, 72°C for 1 min, followed by one cycle of 72°C for 10 min and 4°C hold. The amplifications were performed on ABI Veriti thermocyclers (Applied Biosystems). After an equimolar pooling of the amplification products, a size-selection process was performed on each pool with Agencourt AMPure XP (Beckman Coulter). A quantification step was performed on size-selected pools using the Qubit 2.0 Fluorometer (Life Technologies). Quantified pools were finally loaded on an Illumina MiSeq 2 × 250 flow cell at 10 pM.

The pipeline processing was done at RTL Genomics. The pipeline philosophy involved a merging step of forward and reverse reads using the PEAR Illumina paired-end read merger (Zhang et al., 2014). Merged reads were then trimmed and sorted from longest to shortest. Reads were then de-replicated using the USEARCH algorithm and clustered at 4% divergence with the USEARCH clustering algorithm (Edgar, 2010). After removing singleton clusters, OTU (Operational Taxonomic Unit) selection was performed with the UPARSE algorithm (Edgar, 2013). Chimera were detected using the de novo mode of the UCHIME chimera detection software (Edgar et al., 2011). Following this detection, each centroid was mapped to its corresponding OTU and tagged as chimeric or non-chimeric. Each read was then mapped to its corresponding non-chimeric cluster using the USEARCH global alignment algorithm (Edgar, 2010). A final correction step was performed between the consensus sequence of a cluster and each other sequenced of that cluster.

Sequences were matched against a database derived from NCBI composed of high-quality sequences. The sequences were submitted

TABLE 1 Main characteristics of the fish samples

Type of fish	Fish species	Common name	Habitat	Origin	Sampling site	Sample ID
Smoked fish ($n = 24$)	*Oreochromis niloticus*($n = 8$)	Nile tilapia	Lake	Local fishery	Market	ON1
					Market	ON2
					Market	ON3
					Market	ON4
					Processing site	ON5
					Market	ON6
					Processing site	ON7
					Processing site	ON8
	Merluccius polli($n = 8$)	Benguela hake	Sea	Imported frozen	Market	MP1
					Market	MP2
					Market	MP3
					Processing site	MP4
					Market	MP5
					Processing site	MP6
					Processing site	MP7
					Market	MP8
	Scomber scombrus($n = 8$)	Atlantic mackerel	Sea	Imported frozen	Market	SS1
					Market	SS2
					Market	SS3
					Market	SS4
					Processing site	SS5
					Processing site	SS6
					Processing site	SS7
					Market	SS8
Smoked-dried fish ($n = 24$)	*Ethmalosa fimbriata*($n = 8$)	Bonga shad	Lake	Local fishery	Market	EF1
					Market	EF2
					Market	EF3
					Processing site	EF4
					Market	EF5
					Market	EF6
					Market	EF7
					Processing site	EF8
	Cypselurus cyanopterus($n = 8$)	Flying fish	Sea	Local fishery	Market	CC1
					Market	CC2
					Market	CC3
					Processing site	CC4
					Processing site	CC5
					Processing site	CC6
					Processing site	CC7
					Market	CC8
	Sphyraena barracuda($n = 8$)	Barracuda	Sea	Local fishery	Market	SB1
					Market	SB2
					Market	SB3
					Processing site	SB4
					Market	SB5

(Continues)

TABLE 1 (Continued)

Type of fish	Fish species	Common name	Habitat	Origin	Sampling site	Sample ID
					Market	SB6
					Processing site	SB7
					Market	SB8

to the NCBI Sequence Read Archive (SRA) under the accession numbers SAMN13889202 to SAMN13889249.

2.4 | Statistical analyses

Analyses were performed using software R (Version 3.6.1). Normality (Shapiro-Wilk) and equal variance tests were performed to inspect the distribution of the OTUs richness and Shannon index, as well as of relative abundance values of the most dominant bacterial phyla and taxa found across the six fish species, sampling sites, and across SF and SDF samples, all estimated from amplicon sequencing data. Variables that showed normal data distribution were tested whether mean values obtained for all sample groups were equal, followed by one-way ANOVA to determine the significance between fish species, sampling sites (market and processing site), and the two sample categories (SF versus SDF). The Kruskal–Wallis test or Mann–Whitney U test was employed in absence of normal distributions. A Newman–Keuls test was used to verify differences among sample categories in a pair-wise manner. A significant difference was established at $p < .05$.

3 | RESULTS

3.1 | General bacterial composition of SF and SDF

A total of 1,731,776 high-quality reads were obtained from the 48 investigated samples. The number of reads per sample ranged from 6,851 to 102,696 with a mean value of 35,745 reads. The number of OTUs (Operational Taxonomic Units) per fish sample ranged from 49 to 689, with a mean of 196 OTUs. Overall, 16 bacterial phyla were recovered, with an overwhelming majority belonging to two major phyla: Firmicutes (43.3%) and Proteobacteria (43.6%). Three additional phyla were well represented: Actinobacteria (6.4%), Fusobacteria (2.3%), and Bacteroidetes (1.2%) (Figure 1). The other phyla had a relatively low abundance <0.5% and accounted together for 0.4% of all reads. The remaining reads were unclassified or unknown at the phylum level and accounted for 0.6% and 2.3%, respectively. OTUs belonging to phyla Actinobacteria, Fusobacteria, and Bacteriodetes were detected in 46/48, 27/48, and 37/48 samples, respectively, while those of Firmicutes and Proteobacteria occurred in all samples.

Within the phyla that form the core bacterial communities, the most abundant families in SF samples were the Moraxellaceae (19.5%), Staphylococcaceae (19.3%), Planococcaceae (15.5%), Leuconostocaceae (7.2%), and Bacillaceae (6.9%), whereas the SDF samples were dominated by Bartonellaceae (19.2%), Staphylococcaceae (12.6%), Enterobacteriaceae (7.5%), and Vibrionaceae (6.8%). (Figure 2, Table 2). Of note is the observation that Bartonellaceae OTUs were present in many more samples of SDF (14/24) than SF (3/24).

Figure 3 shows the composition at the genus level of bacterial communities in SF and SDF samples. The number and relative abundance of genera differed among samples of the same fish species. A total of 384 distinct genera were detected across all samples, with 17 genera present in at least 31 of the 48 samples (> 60%), namely *Acinetobacter*, *Aerococcus*, *Bacillus*, *Clostridium*, *Corynebacterium*, *Enterobacter*, *Enterococcus*, *Kocuria*, *Kurthia*, *Lactococcus*, *Macrococcus*, *Photobacterium*, *Propionibacterium*, *Psychrobacter*, *Pseudomonas*, *Staphylococcus*, and *Weissella*.

In SF samples, the number of bacterial genera ranged from 20 to 79 in *O. niloticus*, 18 to 96 in *M. polli*, and 22 to 68 in *S. scombrus*. Similarly, the composition of the bacterial communities varied greatly across the SDF samples: from 15 to 139 genera in *C. cyanopterus*, 47 to 120 in *E. fimbriata*, and 25 to 152 in *S. barracuda*. As anticipated *Bartonella* spp. were identified in all three SDF species with its highest relative abundance in *E. fimbriata* samples (28.6% on average).

3.2 | Effect of fish product, sampling site, and fish species on bacterial communities

Based on OTU data, the sample richness and diversity (Shannon index) were estimated (Figure 4). Shannon index ranged from 0.5 to 4.5 in SF samples and from 0.4 to 5.7 in SDF samples. Similarly, the richness ranged from 49 to 269 in SF samples and from 50 to 689 in SDF samples. When comparing the Shannon index and richness across the six fish species, no significant difference ($p > .05$) was observed (Figure 4a,d). Similarly, no significant difference ($p > .05$) was observed between the Shannon index and richness of SF versus SDF (Figure 4b, e), or between the Shannon index of fish samples collected in markets versus processing sites (Figure 4c). The only significant difference ($p < .05$) was observed for the richness of samples collected in markets versus those collected from processing sites (Figure 4f). Likewise, fish samples from the market have a relative abundance of *Bacillus*, *Bartonella*, *Enterobacter*, *Macrococcus*, *Morganella*, and *Weissella* higher than those from processing plants (data not shown).

The effect of the type of process on the occurrence of the diverse taxa was assessed by comparing their relative abundance in the two types of fish products (SF versus SDF). The abundance of OTUs belonging to *Firmicutes* in SF was significantly higher ($p < .05$) than

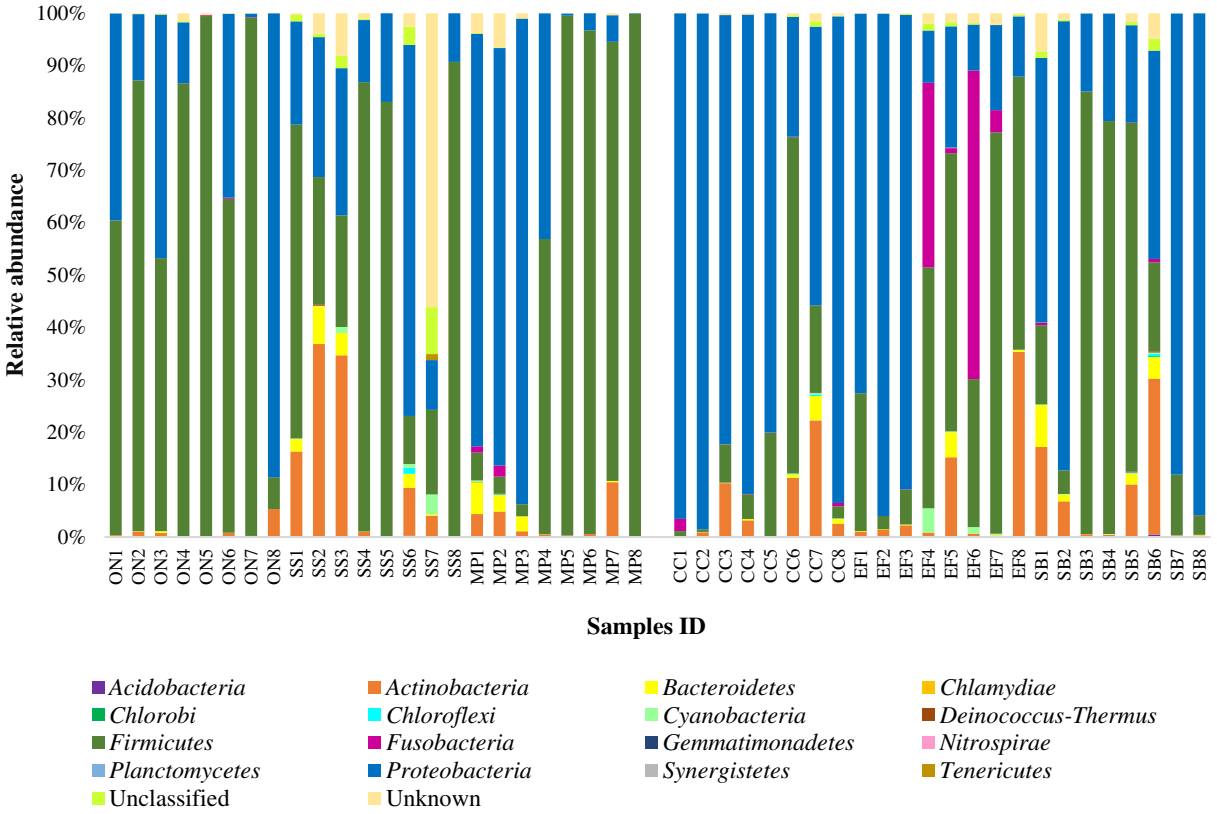

FIGURE 1 Bacterial taxonomic composition of individual fish sample, at the phylum level, as determined by high-throughput sequencing. The fish names were abbreviated as follows: ON, SS and MP for the smoked *O. niloticus*, *S. scombrus* and *M. polli*, respectively, and CC, EF and SB for the smoked-dried *C. cyanopterus*, *E. fimbriata* and *S. baraccuda*, respectively

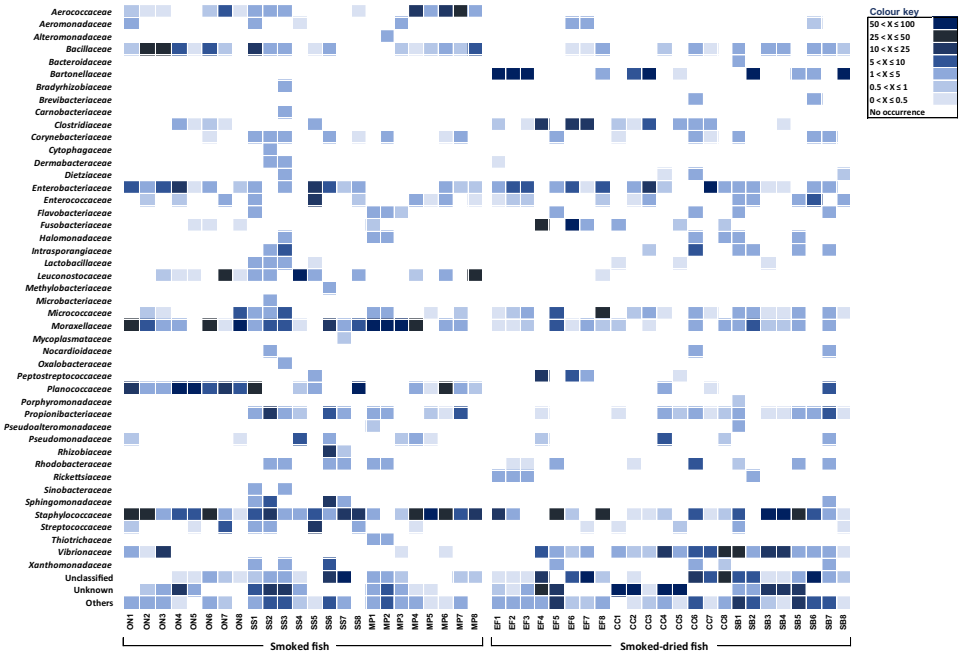

FIGURE 2 Heatmap showing the relative abundance of bacterial families in 48 fish samples from different origins, as determined by high-throughput sequencing. The color codes indicate the range of relative abundance for a given taxa. Fish abbreviations are as in Figure 1

that in SDF. On the contrary in SDF, the abundance of OTUs from Proteobacteria and Fusobacteria were significantly higher ($p < .05$) than those recovered in SF (Table 2). The relative abundance of OTUs belonging to the Aerococcaceae, Bacillaceae, Leuconostocaceae, Moraxellaceae, Planococcaceae, Sphingomonadaceae, and Staphylococcaceae families were significantly higher ($p < .05$) in SF than in SDF samples, while Bartonellaceae, Clostridiaceae, Enterobacteriaceae, Fusobacteriaceae, and Vibrionaceae were significantly higher ($p < .05$) in SDF. At the genus level (Table 3), the relative abundance of *Aerococcus*, *Bacillus*, *Kurthia*, *Macrococcus*, *Psychrobacter*, and *Weissella* were significantly higher ($p < .05$) in SF than in SDF, while the relative abundances of *Clostridium*, *Photobacterium*, and *Vibrio* were significantly higher ($p < .05$) in SDF. The environment of capture (Sea versus lake) also has an influence on fish bacterial diversity. In this study, OTUs corresponding to the genus *Photobacterium* were detected in sea fish with a relatively higher abundance than in lake fish (on average 5.3% versus 1.1%). Other genera such as *Salinicoccus*, *Marinobacter*, and *Marinobacterium* were detected only in sea fish. No significant difference ($p > .05$) was observed between the relative abundance of bacterial genera of fish from market versus processing site (data not shown).

4 | DISCUSSION

This study explored the bacterial diversity in SF and SDF samples using high-throughput sequencing of the 16S rRNA gene on the Illumina MiSeq platform. The results indicated that *Proteobacteria* and *Firmicutes* are the most abundant bacterial phyla, both in SF and SDF samples. Notably, an important variability was observed in the bacterial communities across and within the different fish samples. Also, the bacterial communities in the fish samples could be subdivided into three main bacterial groups: ubiquitous, associated with aquatic environment and of human or animal origins.

4.1 | Variation in the composition of bacterial community in smoked fish products

Shannon diversity index and richness showed a great variability between samples. However, no significant difference was observed for Shannon diversity index and richness between SF and SDF. Interestingly, fish samples collected from markets had a greater OTU richness than those collected from processing sites (Figure 4f).

TABLE 2 Relative abundance (%) of the most frequent bacterial phyla and families in SF versus SDF

	SF ($n = 24$)		SDF ($n = 24$)		
	Mean	Min–Max	Mean	Min–Max	*p*-value
Phylum					
Actinobacteria	5.5[a]	0.0–36.9	8.6[a]	0.1–35.3	0.25
Bacteriodetes	1.2[a]	0.0–7.2	1.5[a]	0.0–4.8	0.29
Firmicutes	57.9[a]	2.3–99.0	38.9[b]	0.5–84.5	0.00
Fusobacteria	0.0[a]	0.0–2.1	7.2[b]	0.0–58.9	0.02
Proteobacteria	30.5[a]	0.0–88.6	41.0[b]	8.8–98.5	0.00
Family					
Aerococcaceae	4.1[a]	0.0–29.0	0.1[b]	0.0–0.4	0.00
Bacillaceae	6.9[a]	0.0–49.8	1.0[b]	0.0–5.0	0.04
Bartonellaceae	0.0[a]	0.0	19.2[b]	0.0–95.1	0.00
Clostridiaceae	0.2[a]	0.0–2.5	2.6[b]	0.0–20.1	0.04
Enterobacteriaceae	2.5[a]	0.0–17.1	7.5[b]	0.0–9.8	0.30
Enterococcaceae	1.4[a]	0.0–12.5	1.0[a]	0.0–9.9	0.84
Fusobacteriaceae	0.2[a]	0.0–2.1	4.3[b]	0.0–58.9	0.04
Leuconostocaceae	7.2[a]	0.0–82.2	0.1[b]	0.0–0.7	0.00
Micrococcaceae	0.8[a]	0.0–5.3	3.0[a]	0.0–34.7	0.08
Moraxellaceae	19.5[a]	0.0–88.2	1.7[b]	0.0–8.8	0.00
Peptostreptococcaceae	0.1[a]	0.0–1.5	0.7[b]	0.0–11.6	0.04
Planococcaceae	15.5[a]	0.0–88.9	0.4[b]	0.0–5.0	0.00
Propionibacteriaceae	2.7[a]	0.0–21.5	1.1[a]	0.0–6.3	0.95
Sphingomonadaceae	0.8[a]	0.0–15.7	0.1[b]	0.0–2.0	0.04
Staphylococcaceae	19.3[a]	0.1–50.6	12.6[b]	0.1–65.3	0.01
Vibrionaceae	1.0[a]	0.0–18.2	6.8[b]	0.0–29.1	0.00

[a,b]For each family, mean values followed by different superscripts on the row indicate that they differ significantly ($p < .05$) between SF and SDF.

Abbreviations: Min, Minimum; Max, Maximum; *n*, number of samples analyzed.

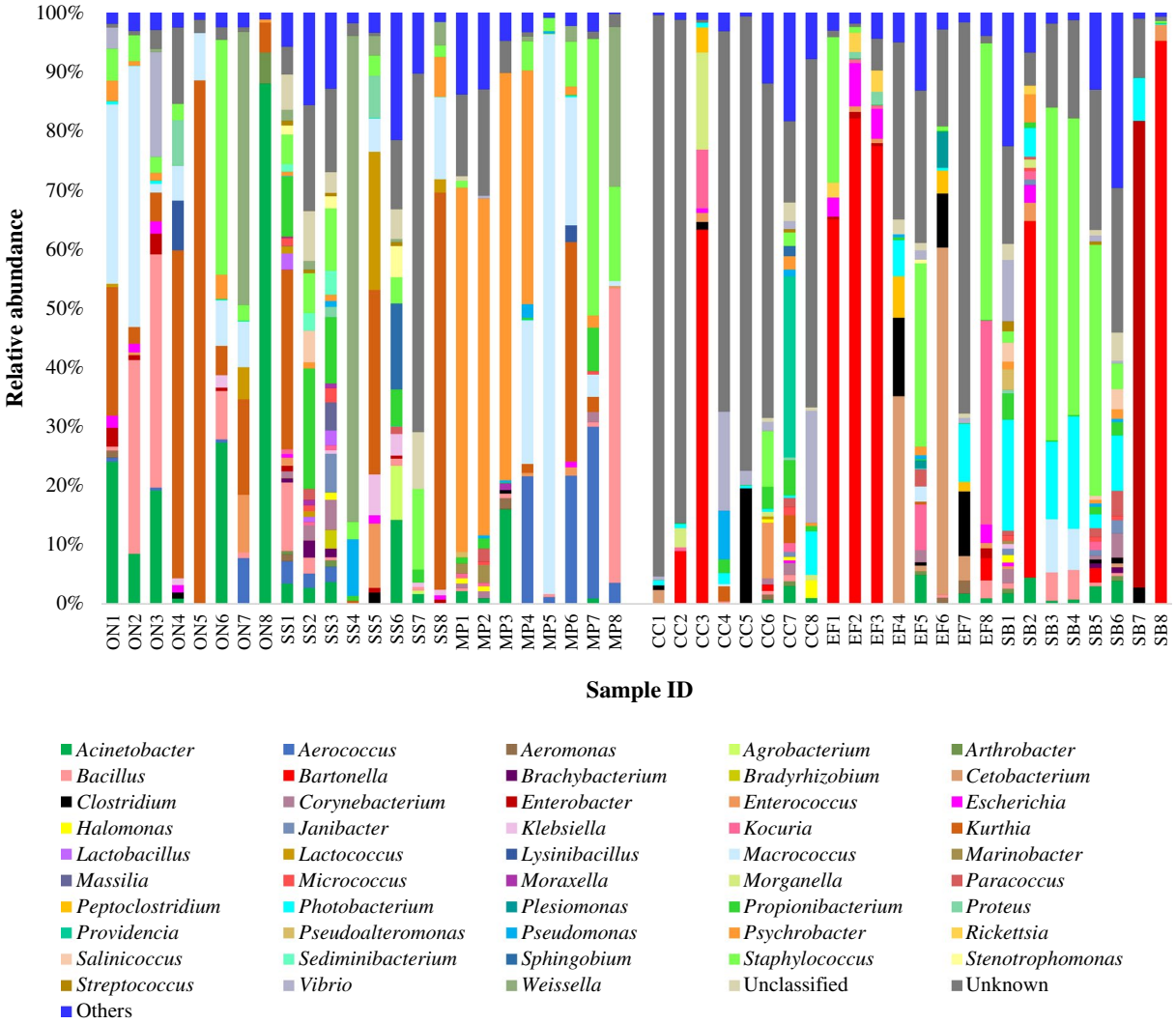

FIGURE 3 Bacterial composition of individual fish sample, at the genus level, as determined by high-throughput sequencing. Fish abbreviations are as in Figure 1

This difference in OTU richness could be explained by the fact that in markets (open air), smoked fish products are constantly exposed to environmental and human (sellers and customers) contamination.

It was also observed that the diversity of bacterial genera identified varies greatly from one fish species to another, and within samples from the same fish species, whereas it might have been expected a similar composition of the bacterial communities within samples of the same species. This important variability likely originates, at least partly, in the diversity of the collecting sites (different markets and processing sites) where the samples were not handled by the same processors or were subjected to different environmental contaminations. Bacterial strains present on the surface (or within) fish can also be affected by fish species and geographical location as previously indicated by several authors (Parlapani, 2021; Parlapani et al., 2018; Pimentel et al., 2017; Zotta et al., 2019). Important inter-individual variation in the composition of bacterial communities among fishes of the same species has also been previously reported (Arias et al., 2019; Boutin et al., 2014). The detection of the genus *Photobacterium* in sea fish with relative abundance higher than in lake fish, and the presence of *Salinicoccus*, *Marinobacter*, and *Marinobacterium* genera exclusively in sea fish is likely due to the fact that these bacteria are halophilic, as previously reported (Chen et al., 2007; Durán-Viseras et al., 2021; Grimaud, 2010).

4.2 | Ubiquitous and aquatic bacteria as part of the SF and SDF microbiomes

Actinobacteria, Firmicutes, Fusobacteria, and Proteobateria were the most abundant phyla in the fish samples. These phyla have been reported as important part of fish microbiome (Arias et al., 2013; Boutin et al., 2014; Zotta et al., 2019), in both natural and aquaculture environments (Green et al., 2013; Navarrete et al., 2009; Smriga et al., 2010). Proteobateria has also been reported as the most common phylum associated with the skin and gill of teleost fish (Llewellyn et al., 2014), including the skin microbiome of sea bass

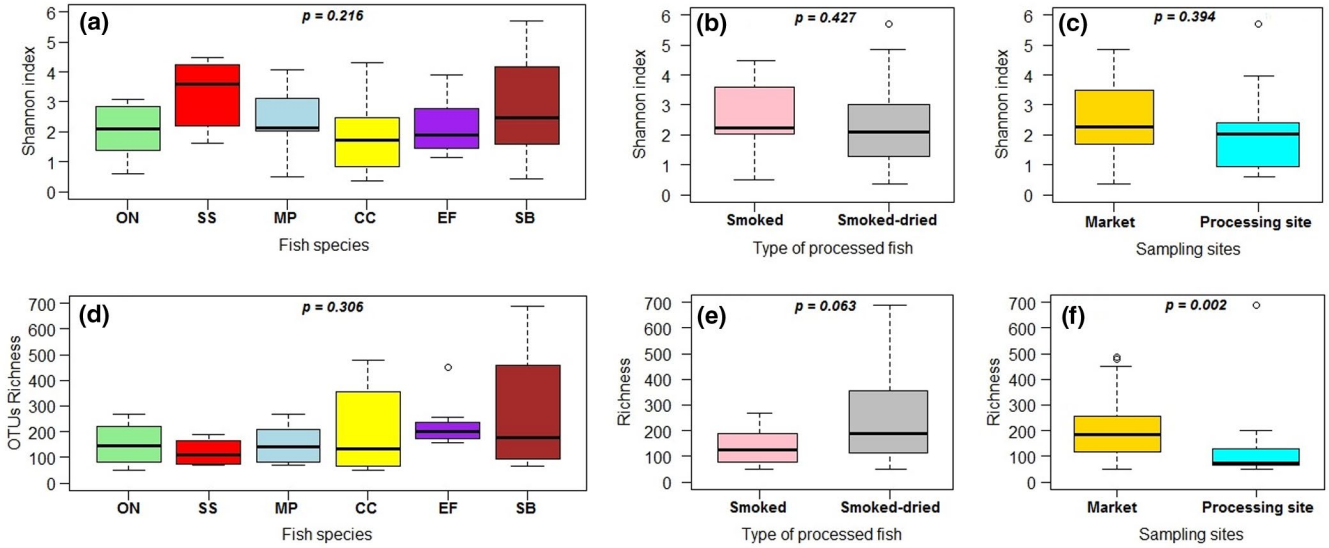

FIGURE 4 Shannon diversity (a–c, top graphs) and richness (d–f, bottom graphs) based on OTUs. The boxes represent the interquartile range (IQR) between the first and third quartile (i.e. 25th and 75th%, respectively), and the horizontal line inside the box defines the median. Whiskers represent the lowest and highest values, within 1.5 times the IQR from the first and third quartiles. Samples with a value for Shannon index or richness exceeding 1.5 times the IQR are displayed as open points above the boxes. $p < .05$ indicates a significant difference. Fish species abbreviations are as in Figure 1

and sea bream (Chiarello et al., 2015; Rosado et al., 2018). Similarly, Arias et al. (2019) have reported Proteobacteria as the most common phylum on skin of *Micropterus salmoides*, *Lepomis macrochirus*, and *Lepisosteus oculatus*, while Parlapani et al. (2018) found Proteobateria and Firmicutes as the most abundant phyla in sea bream flesh. These two bacterial phyla were also noticed as dominant in *hongoe*, a traditional Korean fermented fish product (Jang et al., 2017; Zhao & Eun, 2020).

The results of our study showed that bacteria from the Bacteriodetes phylum were poorly represented. This observation agrees with those of previous studies showing a low occurrence of Bacteroidetes on skin of several fish species (Boutin et al., 2014; Tapia-Paniaga et al., 2018). Yet, others studies have claimed Bacteroidetes as the predominant phyla on skin of fish of various species (Chiarello et al., 2015; Lowrey et al., 2015; Rosado et al., 2018).

At family level, Aerococcaceae, Bacillaceae, Bartonellaceae, Enterobacteriaceae, Leuconostocaceae, Moraxellaceae, Planococcaceae, Propionibacteriaceae, and Staphylococcaceae were predominant. These microorganisms are found in a wide range of ecosystems, including aquatic environments, human body, and various food products (Anihouvi et al., 2019; Grice & Segre, 2011). These different phyla and families are observed in varying proportions between SF and SDF. This can be explained at least partly by the diversity of sample collection sites as mentioned above; but also by the fact that each category of fish (SF and SDF) would behave like an ecological niche favorable to some particular groups of microorganisms.

Bacterial genera with an average relative abundance ≥0.1% across all the analyzed samples consisted of a variety of Gram-negative (52%) and Gram-positive (48%) bacteria. The Gram-positive bacteria significantly represented in our samples were *Aerococcus*, *Bacillus*, *Clostridium*, *Corynebacterium*, *Kocuria*, *Kurthia*, *Macrococcus*, *Propionibacterium*, *Staphylococcus*, and *Weissella*. As for Gram-negative bacteria, notably *Acinetobacter*, *Aeromonas*, *Photobacterium*, *Pseudomonas*, and *Vibrio*, their presence in an aquatic environment and typical association with fish (Ayeloja et al., 2018; Ikutegbe & Sikoki, 2014; Izuchukwu, 2017; Urbanczyk et al., 2011) was also observed in the present study. Members of these genera have regularly been found associated with spoiled fish and fish products (Kuuliala et al., 2018; Parlapani et al., 2018). Other Gram-negative bacteria such as *Psychrobacter* spp. or *Pseudomonas* spp. are known to play an important role in urea decomposition, medium-chain lipid breakdown, and hydrolysis of amino acids, with production of several volatile compounds including 1,3-butanediol, carbon disulfide, H_2S, 2-pentanamine, and ammonia in fish and fish product (Zhao & Eun, 2020). Some of these compounds participate to the unpleasant odors of spoiled fish, as reported by Assogba et al. (2019).

Considering the food safety point of view, OTUs of bacterial genera with pathogenic potential have been detected. These included *Vibrio* spp., such as *Vibrio cholerae* (OTU 1617), the causative agent of pandemic diarrheal diseases in humans (Chourashi et al., 2016). Interestingly, OTUs classified as *Klebsiella pneumoniae* (OTU 425), *Morganella morganii* (OTUs 221, 938, and 1,024), *Photobacterium phosphoreum* (OTU 1,287), and *Photobacterium damselae* (OTU 800, 842, 934) were also found. These bacteria are known to exhibit histidine decarboxylase activity that can lead to the production of histamine in fish and fish products (Barcik et al., 2017; Feng et al., 2016). Iko Afé et al. (2021) has reported a high amount of biogenic amines (up to 4,400 mg/kg), with histamine content (up to 501.5 mg/kg) in Benin SF products which is above the acceptable

TABLE 3 Relative abundance (%) of the most frequent bacterial genera in SF versus SDF

	SF ($n = 24$)		SDF ($n = 24$)		
Genus	Mean	Min–Max	Mean	Min–Max	*p*-value
Aerococcus	4.1[a]	0.0–29.5	0.0[b]	0.0–0.3	0.00
Aeromonas	0.2[a]	0.0–1.8	0.2[a]	0.0–2.1	0.79
Bacillus	6.3[a]	0.0–49.8	0.8[b]	0.0–5.0	0.01
Bartonella	0.0[a]	0.0–0.1	19.2[b]	0.0–95.3	0.00
Clostridium	0.2[a]	0.0–1.9	2.6[b]	0.0–19.6	0.00
Enterobacter	0.5[a]	0.0–3.5	3.5[a]	0.0–79.0	0.61
Enterococcus	1.1[a]	0.0–10.9	0.8[a]	0.0–9.4	0.61
Escherichia	0.4[a]	0.0–2.1	1.0[a]	0.0–7.3	0.45
Klebsiella	0.7[a]	0.0–6.9	0.1[a]	0.0–0.8	0.06
Kocuria	0.2[a]	0.0–0.8	2.5[a]	0.0–34.5	0.07
Kurthia	15.5[a]	0.0–88.6	0.4[b]	0.0–4.7	0.00
Macrococcus	11.3[a]	0.0–94.8	0.8[b]	0.0–9.0	0.00
Micrococcus	0.3[a]	0.0–2.4	0.3[a]	0.0–1.3	0.38
Moraxella	0.1[a]	0.0–1.1	0.0[a]	0.0–0.2	0.37
Photobacterium	0.1[a]	0.0–0.5	4.4[b]	0.0–19.0	0.00
Propionibacterium	2.6[a]	0.0–20.4	1.0[a]	0.0–5.9	0.66
Psychrobacter	10.4[a]	0.0–68.9	0.5[b]	0.0–4.7	0.02
Staphylococcus	7.7[a]	0.0–46.8	11.4[a]	0.0–56.3	0.14
Vibrio	0.9[a]	0.0–17.6	2.3[b]	0.0–19.0	0.00
Weissella	7.3[a]	0.0–88.2	0.1[b]	0.0–0.4	0.00

[a,b]For each genus, mean values followed by different superscripts indicate that they differ significantly ($p < .05$) between SF and SDF. Min, minimum; max, maximum; *n*, number of samples analyzed.

limit of 100–200 mg/kg set by Commission Regulation (2005). The consumption of fish containing a high content of histamine can cause various health disorders to the consumers, including facial itching, torso or body rash, nausea, vomiting, diarrhea, tachycardia, hypotension, respiratory distress and, in rare cases, may result in death (Guergué-Díaz de Cerio et al., 2016). The implementation of good hygiene practices during fish handling is of primary importance to avoid the contamination of fish by histidine decarboxylase-producing bacteria.

4.3 | Bacteria resulting from human or animal contamination

Several bacteria that originate from contamination by human or animal have also been detected in this study. This is the case for the Enterobacteriaceae family and the *Bartonella*, *Propionibacterium*, and *Staphylococcus* genera. Enterobacteriaceae are found in a wide range of environments, mostly in intestine of human or animals. Although some of these bacteria might have been killed during the hot smoking as practiced in Benin, Anihouvi et al. (2019) have reported a high density of living Enterobacteriaceae in 23 of the SF and SDF samples used in this study, as evidence of post-smoking contamination. This family of bacteria may also contain germs responsible for hydrogen sulfide, cheesy and ammonia-like off-odors/flavors (Ghaly et al., 2010; Nychas et al., 2008), or behaving as pathogens (Fall et al., 2019). Similarly, bacteria pertaining to the *Acinetobacter*, *Bacillus*, *Enterobacter*, *Enterococcus*, *Escherichia*, *Klebsiella*, and *Staphylococcus* genera are often associated with human contamination which results from inadequate attention to hygiene during handling (Parlapani et al., 2020), affecting fish products quality and safety.

OTUs belonging to *Escherichia coli* (OTU 409, 736, 2,673) and *Clostridium perfringens* (OTU 306, 311, 641, 1662, 2,375) were also found in the samples, albeit at relatively low abundance. This observation is the evidence of contamination by faecal matter from human or animal origin, as recently noted by Anihouvi et al. (2019) who reported low densities of living *E. coli* and *C. perfringens* in the SF and SDF samples investigated in this study. Regarding the *Bacillus* genus, OTUs 1,056 and 2,201 pertain to the *B. cereus* species known to include potential diarrheic or emetic strains (Griffiths & Schraft, 2017). Finally, in agreement with the findings of Anihouvi et al. (2019) based on culture-based approaches, our data confirmed the absence (no detection) of OTUs related to the pathogenic *Salmonella* spp., *Staphylococcus aureus*, or *Listeria monocytogenes* in the fish samples. The non-detection of these bacteria is a positive observation in the context of food safety, as they are frequently involved in food-borne illness outbreak worldwide (Ciupescu et al., 2018; Hennekinne et al., 2015).

The *Bartonella* genus has been reported to originate from rats, cats, or dog (Jiyipong et al., 2015). Samples that contained this bacterium were probably exposed to these animals during processing, storage, or selling, as observed in street food markets by Anihouvi et al. (2020). Similarly, the presence of *Propionibacterium acnes* (OTU 1,053), *Propionibacterium granulosum* (OTU 1794), and *Staphylococcus epidermidis* (OTU 951) were also detected. To avoid all these human contaminations, processors should apply good hygiene and handling practices during processing and especially after the cooking step, and limit excessive handling of the products by customers at selling place.

5 | CONCLUSION

The application of 16S rRNA amplicon sequencing revealed a large number of bacterial strains associated with smoked and smoked-dried fish. Among the bacteria identified, some inhabit aquatic environments, while others originated from human, animal, and environmental contamination during processing and selling. While some bacteria identified may participate in the spoilage of fish products, others have a negative impact on their harmlessness, making these food potential sources of foodborne illnesses. With a dual objective of reducing food losses induced by spoilage of these fish products, and preserving the health of the consumer, it is necessary to find methods to limit the occurrence and proliferation of these bacteria in SF and SDF. This could be done through the application of good handling practices for freshly caught fish on boats, but also via the application of good manufacturing and hygiene practices during food processing and selling.

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CONFLICT OF INTEREST

There is no conflict of interest among authors: all authors agreed that this manuscript is submitted to *Journal of Food Processing and Preservation*.

AUTHOR CONTRIBUTION

Dona G. H. Anihouvi: Conceptualization; Data curation; Investigation; Methodology; Validation; Visualization; Writing-original draft; Writing-review & editing. **Olivier Henriet:** Conceptualization; Methodology; Validation; Writing-review & editing. **Yénoukounmè Euloge KPOCLOU:** Writing-review & editing. **Marie-Louise SCIPPO:** Writing-review & editing. **Djidjoho Joseph HOUNHOUIGAN:** Writing-review & editing. **Victor Bienvenu ANIHOUVI:** Writing-review & editing. **Jacques MAHILLON:** Conceptualization; Methodology; Resources; Supervision; Validation; Writing-review & editing.

AUTHOR CONTRIBUTIONS

Dona Gildas Hippolyte Anihouvi: Conceptualization, Methodology, Investigation, Data curation, Validation, Visualization, Writing-original draft, Writing-review & editing; Olivier Henriet: Conceptualization, Methodology, Validation, Writing-review & editing; Yénoukounmè Euloge Kpoclou: Writing-review & editing; Marie-Louise Scippo: Writing-review & editing; Djidjoho Joseph Hounhouigan: Writing-review & editing; Victor Bienvenu Anihouvi: Writing-review & editing; Jacques Mahillon: Conceptualization, Methodology, Resources, Supervision, Validation, Writing-review & editing.

ETHICAL GUIDELINES

Ethics approva was not required for this research.

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