A Survey of Microbial Communities on Dry-Aged Beef in Commercial Meat Processing Facilities

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Abstract: Many artisanal meat professionals believe that the microbial populations on the outer crust of dry-aged beef contribute to variation in sensory profiles; however, to date there is minimal information about the microbes themselves that grow on commercially produced dry-aged beef. The microbiome of dry-aged beef bone-in strip loins (Institutional Meat Purchase Specifications #175) from 5 commercial dry aging facilities, including one utilizing ultraviolet light treatment, were surveyed to assess the microbial populations residing on and within each subprimal. Each strip loin was sampled at multiple spatial locations and depths, and the microbial sequences present in the samples were identified using a next-generation sequencing approach. Insufficient microbial DNA was isolated from ultraviolet-light-treated strip loins, indicating that this treatment eliminates all or most microbial growth on the meat. Sequencing results indicated that each establishment was producing meat with different microbial communities, based on Permutational Multivariate Analysis of Variance ($P < 0.01$) and clustering in the Principal Coordinates Analysis plot of Jaccard distances. The position on strip loins from which samples were taken had negligible influence on microbial community structure. Aging facility, and the relative unique environmental conditions within, was determined to be the only observed driver of community structure. Notable operational taxonomic units (OTUs) detected included the spoilage-associated bacterium *Pseudomonas fragi* and the fungal species *Debaryomyces udenii* and *Penicillium polonicum*. An OTU identified as *Mucor* sp. PG272 was found to be present in over 75% of all samples. This OTU may represent a species similar to *Thamnidium*, a mold that has been associated with product quality. This study established a general core microbiome for dry-aged beef observed in commercial facilities, variations of which may—as future research could indicate—contribute to distinct sensory properties.

Introduction

Quality and consistency are two critical aspects of food production, especially in the meat industry. Producers and meat scientists often strive to maximize consistency in order to provide consumers with an equally positive eating experience each time they consume the same product. However, with artisanal products such as dry-aged beef, it can be more challenging to maintain a consistent sensory profile that can regularly match consumers' expectations. Dry aging beef is a practice used by high-end food service restaurants and upscale meat markets to create valued products with distinct flavors and improved tenderness (Savell, 2008). Dry aging involves holding meat for an extended period of time in refrigerated temperatures without protective

packaging. This extended storage time allows proteolytic enzymes to function, resulting in more tender beef (Campbell et al., 2001; Khan et al., 2016). More importantly, the dry aging process has the potential to create unique flavors that other post-harvest techniques (e.g., wet aging) cannot impart.

In comparison, wet aging is a more common technique used in the meat industry. Unlike dry aging, wet-aged product is vacuum packaged before being held in refrigerated temperatures for an extended period of time (Campbell et al., 2001; Khan et al., 2016). Similar to dry aging, wet aging also results in an improvement in tenderness (Jeremiah and Gibson, 2003); however, even this process cannot replicate the flavors associated with dry-aged beef. Nonetheless, because wet aging is more economically viable, produces less unusable trimmings, requires less time and capital investment, and results in a similar improvement in tenderness (Dashdorj et al., 2016), it is a more common practice in the industry to date.

There is little understanding about how unique flavors develop in dry-aged beef. One common theory is that the process of dry aging allows moisture loss and consequently concentrates "beef" flavors (Warren and Kastner, 1992; Campbell et al., 2001; Savell, 2008; Dashdorj et al., 2016) to create a unique and highly palatable product. After 30 d of aging, dry-aged products often lose approximately 15% of their weight through evaporative moisture loss (Dashdorj et al., 2016). This moisture loss often causes a hardened microbe-laden crust to form on the outer surface of the meat product, which is trimmed away and discarded during fabrication and processing. Even though the outer crust layer is trimmed away, some meat specialists theorize that mold growth on the crust contribute to the unique flavors associated with whole-muscle, dry-aged beef. The crust generally has visual microbial growth in the form of patches of mycelia that are not considered a contamination (Brooks and Hansford, 1923) but rather a key aide in enhancing the dry aging process.

While there have been a small number of studies on the dry-aged beef microbiome (Ryu et al., 2018), there is a general dearth of peer-reviewed, published scientific research on the microbiology of the crust. Industry knowledge suggests that a major surface colonizer in dry-aged beef is a fungus or group of fungi in the genus *Thamnidium*, within the phylum Zygomycota (Brooks and Hansford, 1923; Cook, 1995). The genus contains multiple fungi and is known to be adapted to cooler conditions. It is also notable for its ability to produce collagenolytic enzymes that help break down connective tissue in the meat and create a more tender texture

(Dashdorj et al., 2016). A variety of molds—including species in the *Aspergillus*, *Cladosporium*, *Mucor*, and *Penicillium* genera, along with the aforementioned *Thamnidium* genus—have been isolated from fresh meat. Several species of *Aspergillus* and *Penicillium* have been associated with spoiled meat (Frisvad, 1988; Williams, 1990). Some yeasts, such as *Pichia*, *Saccharomyces*, and *Debaryomyces*, are also found to commonly occur in aged meat products.

The relative novelty and small-scale production of dry-aged beef often anecdotally results in an inconsistent sensory profile among products that are intended to be the same. If the microbial populations are able to alter sensory attributes of the meat, then differences in the microbial load may partially explain the inconsistent flavor profiles found in whole-muscle, dry-aged products. This project aimed to survey the general microbial communities found on dry-aged beef in commercial meat production facilities and the consistency or inconsistency of community compositions across and within the product's crust.

Materials and Methods

Beef strip loins

Beef bone-in strip loins (Institutional Meat Purchase Specifications #175) ($n = 2$) were obtained from 5 commercial dry aging facilities across the United States (Locations A, B, C, D, and E). Strip loins used in the study were identified by meat production professionals as having "Moderate" marbling as indicated by the US Department of Agriculture (USDA) beef marbling standards and met all requirements for the USDA G-1 certified beef program (USDA, 2019). Bone-in strip loins are commonly used in industry as well as have been previously used as a dry aging beef model in earlier studies (Laster, 2007, Savell, 2008, DeGeer et al., 2009). Each strip loin was aged under conditions relative to the individual commercial facility for 45 d prior to collection (Table 1).

Table 1. Temperature and relative humidity data for each dry-aging location

	Location				
	A	B	C	D	E
Daily minimum temperature, °C	1.3	1.2	1.4	0.3	1.7
Daily maximum temperature, °C	2.8	7.0	1.9	4.4	4.1
Average temperature, °C	2.1	4.1	1.7	2.4	2.9
Relative humidity, %	84.1	75.9	91.0	n/a	89.9

Unfortunately, a shipping error occurred with Location B, and therefore only a single strip loin was obtained from that location. No antimicrobial interventions beyond temperature control were used in the dry aging facilities with the exception of ultraviolet (UV) lights in Location A as a sort of negative control.

Sampling

Samples from each strip loin were taken from 3 positions on the anterior surface of the *longissimus lumborum et thoracis*: near the subcutaneous fat (superficial), the center of the ribeye surface (medial), and near the 13th rib (deep; Figure 1). Within each position of the strip loin, three 1-cm cubic sections were excised with a scalpel. Each cubic section was cut parallel to the meat surface to create one sample of the outermost rind and one sample of the inner portion. This resulted in 3 biological replicates for each sample from each section ($n = 2$; outer section and inner section) and position on the strip loin ($n = 3$; superficial, medial, and deep). As this was repeated
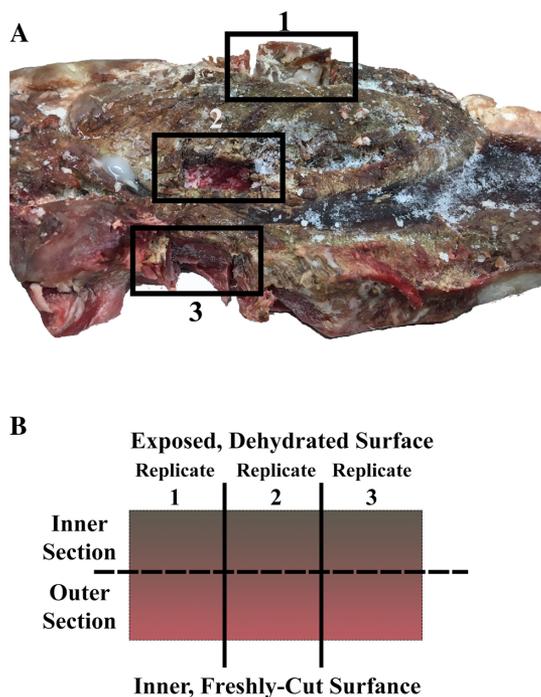
Figure 1. Diagram of sampling on the dry-aged beef strip loin. (A) Boxes depict 3 sampling positions on the crust of the beef loin. The superficial sampling position (position 1) was in lean tissue near the subcutaneous fat layer, the medial sampling position (position 2) was in the center of the loineye muscle, and the deep sampling position (position 3) was in lean tissue nearest the rib bone. (B) Each sampling position was then divided in half to obtain a sample from the outer section of the loin and the inner section of the loin. Each inner and outer section was then divided into thirds to produce 3 replicate samples.

for 9 strip loins, the total number of samples was 162. Samples were taken using sterile techniques in a biosafety cabinet to reduce environmental microbial contamination.

DNA extraction, processing, and analysis

Genomic DNA was extracted from the samples using the Zymo Research Quick-DNA Fungal/Bacterial Kit (Zymo Research Corporation, Irvine, CA). Presence of microbial DNA was evaluated for quantity and quality using the Nanodrop ND-1000 Spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE). The samples were normalized to a concentration of 5 ng/μL. In addition to samples extracted from beef tissue, included in the samples sent for sequencing were a fungal mock community to serve as positive controls as well as a neutral sample containing only extraction buffer to serve as a negative control. The mock community was created by combining equimolar amounts of DNA extracted from known cultures of fungi. The internal transcribed spacer (ITS) and 16S amplicon libraries were sequenced on the MiSeq sequencing platform (Illumina, San Diego, CA) using a 300PE MiSeq sequencing kit and the standard Illumina sequencing primers at the Molecular and Cellular Imaging Center in Wooster, Ohio. The V4–V5 hypervariable region of the bacterial 16S ribosomal RNA gene and the ITS1 region of the fungal nuclear DNA were amplified using universal primers (515F/806R for 16S and ITS1F/ITS2 for ITS) modified to include degenerate bases for maximal inclusiveness (Li et al., 2014; Smith and Peay, 2014). Image analysis, base calling, and data quality assessment were performed on the MiSeq instrument. Resultant data were downloaded from the Illumina BaseSpace hub and processed with the QIIME program (version 1.9.1). Chimeric sequences were removed using UCHIME2 (Edgar et al., 2011), and sequences with Phred scores below 21 were removed with QIIME. Open-reference operational taxonomic unit (OTU) picking was performed with a 97% identity threshold. Taxonomic identification was performed using the UNITE fungal database (version 7) for ITS sequences (Kõljalg et al., 2005) and the Greengenes database (version 13_8) for 16S sequences (DeSantis et al., 2006). Reads aligning with nontarget kingdoms and singleton reads were filtered from the dataset using QIIME. Alpha diversity metrics calculated in QIIME included the Shannon, Simpson (1-D), and Chao1 indices as well as Good's Coverage and unique OTUs.

Statistical analyses

As the objective of the study was to survey the microbiome of commercially produced dry-aged beef, no predictive modelling was conducted considering the small sample sizes. Differences in alpha diversity values were compared using a nonparametric two-sample t-test through the compare_alpha_diversity script in QIIME. Beta diversity was calculated in QIIME using abundance-weighted Jaccard distances between samples and was visualized with two-dimensional Principal Coordinates Analysis plots. Comparisons between communities were assessed using a homogeneity of dispersion test (PERMDISP) to determine homogeneity of variance paired with Permutational Multivariate Analysis of Variance (PERMANOVA) (permutational, $n = 999$) to determine differences between communities by the selected variables in the study. Significance was determined at $P < 0.05$. Bar charts showing community compositions were calculated by mean relative abundance and created in Microsoft Excel 2008 (version 12.3.6). OTU taxonomy assignments were shown at the lowest rank of taxonomy until the genus level provided by the database. Low-abundance OTUs ($< 0.5\%$) were collapsed under "Other" for simplification purposes.

Results and Discussion

After the 45-d aging period, the strip loins had an average intramuscular fat percentage of 6% ($\pm$ 2%) and average moisture levels of 65% ($\pm$ 2.5%).

Amplification of DNA from UV-light-treated aged beef strip loins

Total DNA was extracted from the samples, and microbial DNA was successfully amplified for each sample from Locations B, C, D, and E. Following 2 extraction and amplification attempts, it was determined that no microbial DNA could be amplified from samples from Location A (Figure 2). The extraction and polymerase chain reaction amplifications were performed blind in regard to which locations had utilized UV light treatment. However, after 2 unsuccessful attempts to obtain fungal DNA from samples from Location A, it was confirmed by collaborators that the aging facility utilizing UV light treatment was Location A. From this, it was concluded that the UV light treatment of aging beef strip loins results in extremely reduced microbial communities that have
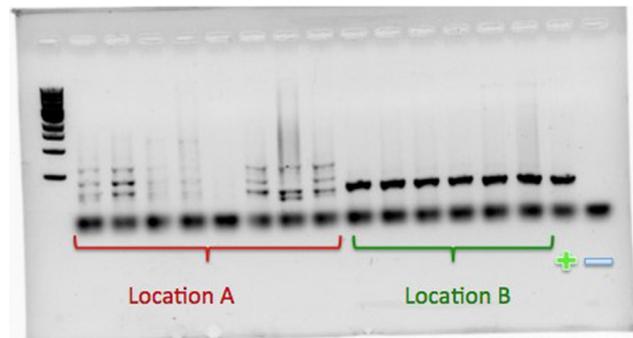
Figure 2. Results of PCR with ITS1F/ITS2 primer pair from Location A and Location B. Samples from Location A, which were aged under ultraviolet light, did not produce a strong band at the expected size (approximately 250 bp), while Location B and the positive control containing a known fungal consortium did yield a clear amplicon band. PCR, polymerase chain reaction

insufficient biomass and thus insufficient DNA to obtain community sequencing data.

Sequencing results

The majority of samples submitted in separate MiSeq runs with fungal and bacterial primers, respectively, underwent successful amplification of the targeted regions (95% and 86% success for fungal and bacterial runs, respectively). Following removal of low-quality reads, chimeric sequences, and sequences from nontarget kingdoms, the fungal ITS run yielded 117 remaining samples with over 2,000 reads. In total, 2,661,548 reads were obtained with a mean of 22,748 reads per sample. From the bacterial 16S data, there were 1,951,150 reads from 109 remaining samples with over 1,800 reads each. The minimum allowed number of reads per sample was lowered for the bacterial dataset to retain as many samples and replicates as possible. The mean number of reads per sample was 17,900, and 217 unique OTUs were identified.

A neutral buffer was included in each run, and a mock community composed of equal amounts of DNA from a diverse group of fungi was included in the fungal run. The neutral controls for the fungal and bacterial runs yielded only 4 and 2 reads, respectively, indicating little to no contamination of the samples in the extraction or sequencing process. The fungal mock community's data showed expected results based on the DNA origins and ratios placed in the mock sample (Figure 3).

Core OTUs

Core OTUs, which are defined here as OTUs appearing in 75% percent or more of all samples with
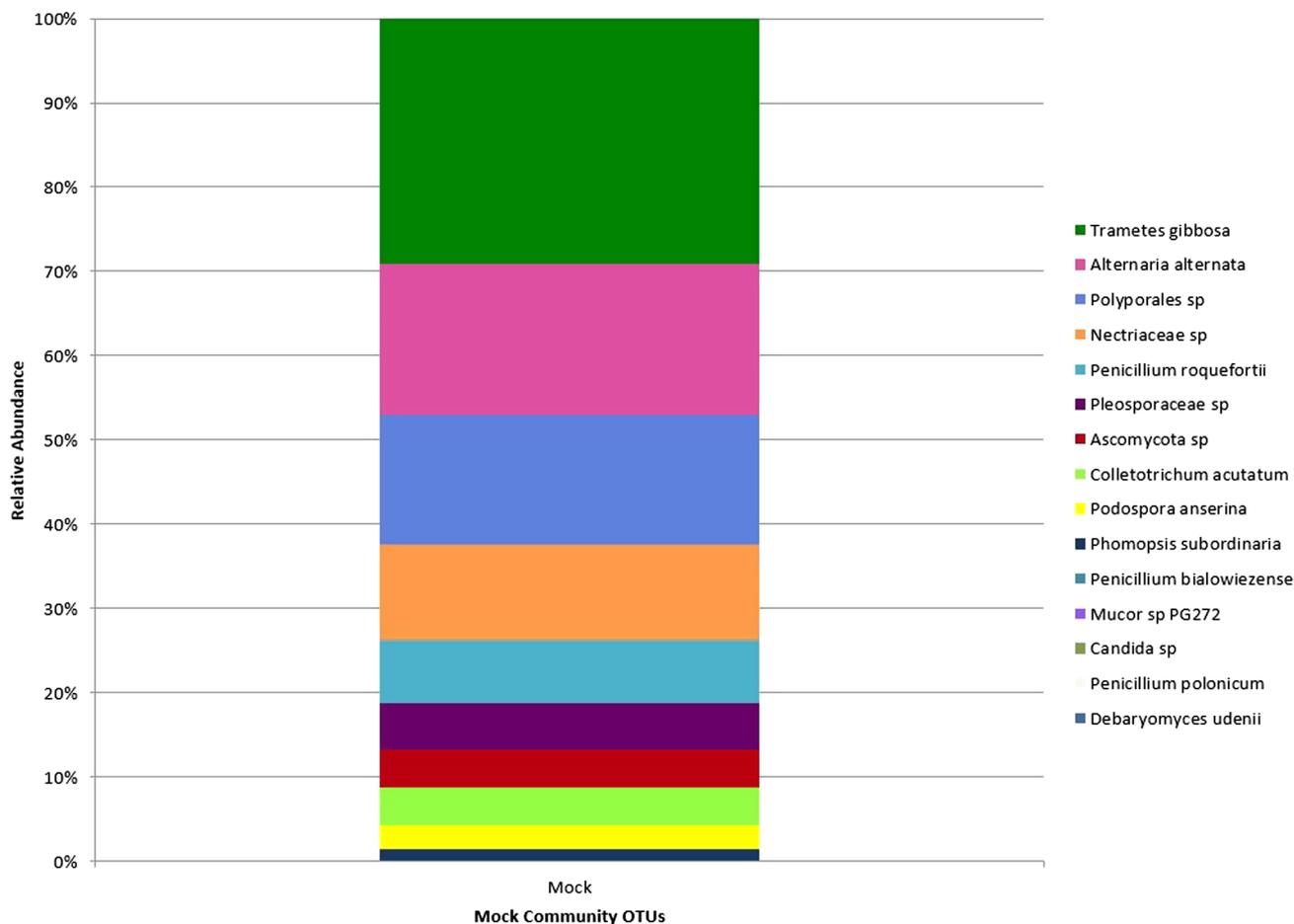
Figure 3. Relative abundances of OTUs identified in the sequenced fungal mock community. OTU, operational taxonomic unit.

a minimum of 10 reads per sample, were assessed for the sample set as a whole (Table 2). Many species that were differentially abundant between locations were determined to be core OTUs, as they were common among the samples, but in varying abundance. It is likely that these fungi and bacteria are commonplace

in dry aging facilities, yet differences in the presence of other competing or co-colonizing microbes as well as differences—small and large—in facility conditions are contributing to the relative dominance of these microorganisms.

Fungal data: Alpha diversity

In total, 120 unique OTUs were identified for all remaining samples in the dataset. The average number of unique OTUs per sample was 118. Good's Coverage (Tables 3 and 4) and rarefaction curves (Figure 4A) approaching an asymptote indicated relatively complete sampling in the ITS run.

The calculated alpha diversity metrics were consistent between samples from each of the 4 sequenced locations. Significant differences ($P < 0.05$) found were between Location B and Locations E, C, and D in the Shannon diversity indices ($P = 0.01$, $P = 0.02$, and $P = 0.02$, respectively), between Locations C and B in the Simpson index ($P = 0.01$), and between Locations B and E ($P = 0.02$) for the number of unique

Table 2. Core fungal and bacterial operational taxonomic units identified in greater than 75% of samples

Percent of Samples[1]	Fungus[2]	Bacteria[3]	OTU Taxonomic Identification
90	X		*Debaryomyces udenii*
90		X	*Pseudomonas fragi*
75	X		*Mucor* PG272
75	X		*Penicillium polonicum*
75	X		*Penicillium bialiowiezense*

[1]The percentage of samples with a minimum of 10 reads for the designated OTU.

[2]An "X" in the fungus column indicates a fungal OTU.

[3]An "X" in the bacteria column indicates a bacterial OTU.

OTU, operational taxonomic unit.

Table 3. Alpha diversity metrics for fungal data obtained from dry-aged beef strip loins by location

| Location	Number of Reads	OTUs[1]	Good's Index	Chao1	Shannon	Simpson
B	16,081	38.306[b]	99.87	65.898[b]	2.016[b]	0.586[b]
C	20,136	42.171[ab]	99.91	74.914[ab]	2.715[a]	0.754[a]
D	23,399	51.112[ab]	99.84	86.543[ab]	2.608[a]	0.682[ab]
E	27,768	47.846[a]	99.92	80.595[a]	2.528[a]	0.685[ab]

[a,b]Values within a column lacking a common superscript differ significantly ($P < 0.05$).

[1]The number of unique OTUs within each location.

OTU, operational taxonomic unit.

Table 4. Alpha diversity metrics for bacteria data obtained from dry-aged beef strip loins by location

Location	Number of Reads	OTUs[1]	Good's Index	Chao1	Shannon	Simpson
B	21,748	59.67[b]	99.75	59.67	2.48[b]	0.477
C	9,557	90.46[a]	99.17	90.46	3.41[a]	0.741
D	11,531	85.64[a]	99.38	85.64	3.15[a]	0.715
E	28,271	71.11[ab]	99.79	71.11	2.553[b]	0.689

[a,b]Values within a column lacking a common superscript differ significantly ($P < 0.05$).

[1]The number of unique OTUs within each location.

OTU, operational taxonomic unit.

observed species (OTUs). These numbers indicated that Location B has a lower level of diversity by some metrics; however, it is not drastically lower than the other locations. This may be in part due to the unavailability of a second loin from this location. As with most microbial studies, additional samples generally equate to more complete sampling and therefore more OTUs. While this may have impacted diversity levels, the quality of the data from the single Location B sample was sufficient to assess a general community composition and perform analysis of within- and between-location comparisons. Although the sample sizes for the current study were relatively small, precedence has been set with previous studies able to identify significant microbial populations using 3 subprimals per treatment (Ryu et al., 2018; Lee et al., 2019), and the current study was indeed able to identify significant microbial populations at the unique treatment locations. Furthermore, the objective of the study was to establish a base population rather than identify true differences, yet differences were able to be observed even with the low sample sizes.

From the fungal data, notable identified OTUs were *D. udenii*, *Mucor* sp. PG272, *P. polonicum*,
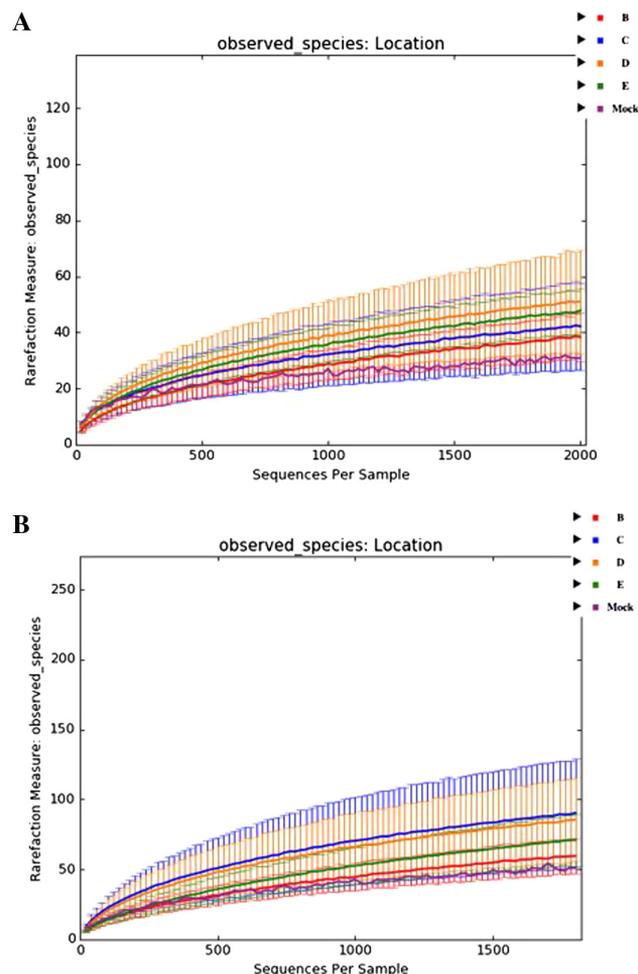
Figure 4. Rarefaction curves for dry-aged beef samples by location, for (A) fungal and (B) bacterial. Bars represent standard deviation. Curves for all locations reach an asymptote indicating adequate sequencing depth.

and *Penicillium bialowiezense* (Figure 5). *D. udenii* is a yeast from the family Saccharomycetaceae (Van der Walt et al., 1989). While there is a lack of information about this particular species—particularly pertaining to any associations with food aging or processing—a similar species, *D. hansenii*, is commonly found in cheeses (Petersen et al., 2002), beer fermentation (Breuer and Harms, 2006), and other aged meats (Jessen, 1995).

Samples from Locations C and D displayed high relative abundances of an OTU identified as *Mucor* sp. PG272. Much like *D. hansenii*, there is a dearth of information available about this particular species. However, this OTU is in the Mucoraceae family and may be closely related to *Thamnidium* spp., another Mucoraceae fungal species known to have associations with the meat aging process and part of the wider group of meat aging fungi colloquially referred to as *Thamnidium*. Fungi in this group are considered by meat industry professionals to play an important role
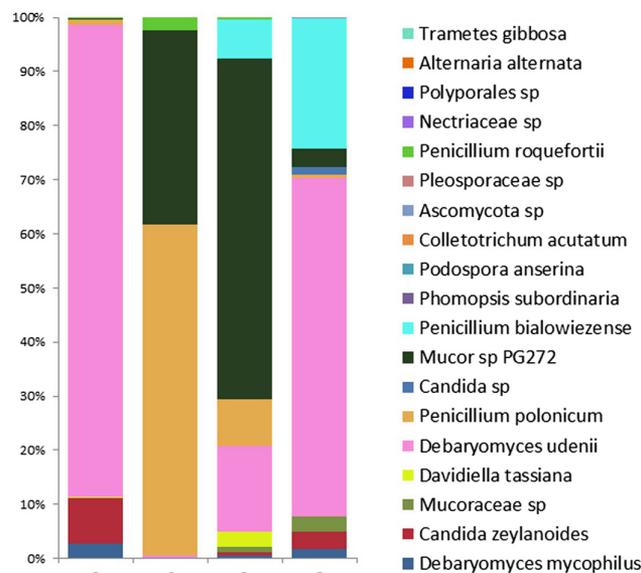
Figure 5. Relative abundances of fungal OTUs from dry-aged beef samples at each location. OTU, operational taxonomic unit.

in the dry aging process through their release of enzymes that break down connective tissue to enhance meat texture and flavor (Dashdorj et al., 2016). A higher abundance of fungi producing these degradative proteases and collagenolytic enzymes may result in a product with more desirable sensory qualities.

Two *Penicillium* species were also found to be highly abundant in the sample set. *P. polonicum* made up 61.07% of reads, on average, from Location C and 8.41% of reads from Location D. It was also present in much smaller proportions (1.04%, 0.66%) of samples from Locations B and E, respectively. This species of *Penicillium* is commonly associated with various meat products (Sonjak et al., 2011). It is most often associated with spoilage rather than desired enzymatic breakdown. Some distinct *P. polonicum* isolates are also associated with the production of verrucosidin (Núñez et al., 2000), a tremorgenic mycotoxin. However, not all isolates of the fungus produce this toxin, nor is it understood whether these isolates can produce the toxin on a meat substrate or at refrigerated temperatures. The detection of this potentially harmful species in large proportions indicates a need for increased targeted safety-testing measures of such artisanal meat products. A second *Penicillium* species, *P. bialowiezense*, was identified in significant quantities in samples from Locations D and E (7.15% and 23.54%, respectively). Unlike *P. polonicum*, *P. bialowiezense* does not have a significant literary indication as a producer of harmful exudates. It is considered a commensal, cosmopolitan, household mold, though it may have some association with fruit spoilage (Nierop Groot et al., 2018). It is possible that
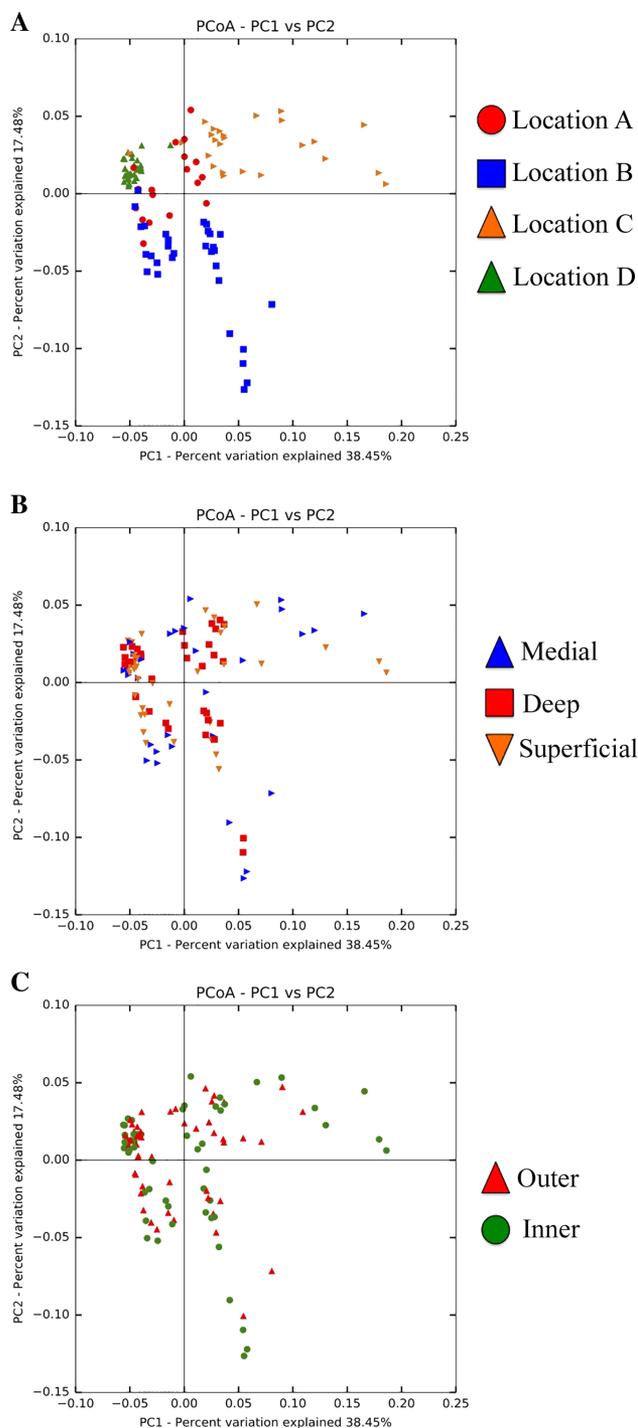
Figure 6. PCoA plots of abundance-weighted Jaccard distances between fungal communities on dry-aged beef by (A) location, (B) position on loin, and (C) section. While strong clustering of the communities is visible for the location variable, there is no distinct visual separation of the samples when sorted by position on loin or section. PCoA, Principle Coordinate Analysis.

the presence of *P. bialowiezense* on dry-aged beef is inert, though its relation to *P. polonicum* may make it a potential target for further investigation in future food safety studies.
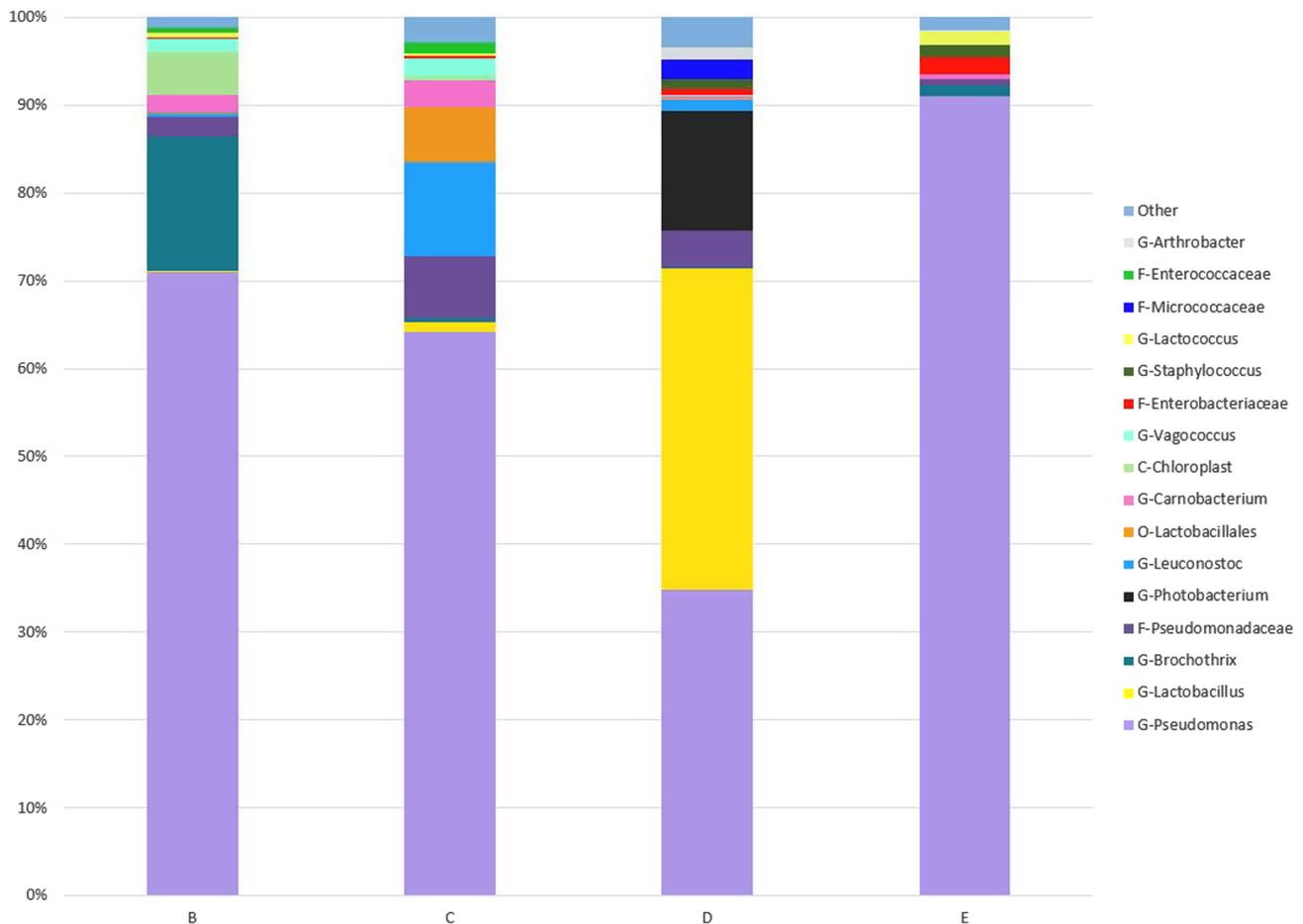
Figure 7. Relative abundances of bacterial OTUs from dry-aged beef samples at each location. OTU, operational taxonomic unit.

Fungal data: Beta diversity

Fungal community composition varied between locations, and the samples show clear clustering by the location variable for the abundance-weighted Jaccard distance metric (Figure 6A). There was no clustering observed for any of the other variables assessed in the study (Figure 6B-6C). PERMDISP analysis showed significant levels of variance in each variable. PERMANOVA (999 permutations) analysis revealed significant differences between the location variables ($P = 0.001$), while the differences between the positions (superficial, medial, and deep; $P = 0.686$) and sections (inner crust and outer crust; $P = 0.57$) were not significant. Based on analysis of the beta diversity calculations and the compositions of the communities assessed through relative abundances, it is likely that the results are a combination of location influence on community structure as well as dispersion within each location variable. The PERMANOVA results indicate that, while the fungal communities are significantly influenced by the facility in which they are aged, the fungi on the loin

are relatively evenly distributed on the surface and to a depth of 1 cm into the meat.

Bacterial data: Alpha diversity

Rarefaction plots of the bacterial sequencing data show high variability in the alpha diversity metrics calculated for the samples within a location. The rarefaction curves (Figure 4B) appear to be approaching an asymptote, and the Good's Coverage Index numbers are high for all locations (Table 3), indicating a relatively complete sampling. There were no significant differences between any of the location treatments for the Chao1 and Simpson diversity indices. For both Observed Species and the Shannon index, Locations C and B were significantly different (calculated by nonparametric two-sample t test with 999 Monte Carlo permutations, $P = 0.01$). Additionally, Location E was significantly different from Locations D and C in only the Shannon index ($P = 0.01$).

In the bacterial dataset, OTUs from the *Pseudomonas* genus composed large proportions of

the reads from each location, with an average of 65.2% relative abundance over all locations (Figure 7). One specific OTU in this genus appeared as a dominant OTU, having a relative abundance of at least 28.5% in each location, with samples from Location B averaging over 60% abundance (Figure 7). While sequencing of only one region of the 16S ribosomal subunit is typically not reliable to provide complete taxonomic assignment to the species rank, it is interesting to note that this prevalent OTU was assigned to the species *P. fragi*. *P. fragi* is a bacterial species commonly associated with the spoilage of dairy products (Hussong et al., 1937; Hebraud et al., 1994). Along with many other *Pseudomonas* species, it is also associated with meat products and varies in terms of impact on meat spoilage and sensory properties depending on the specific isolate present (Ercolini et al., 2010). In sample E, a different OTU assigned to the genus *Pseudomonas* had a relative abundance of 27.98%. The same OTU was present in other locations, but at much lower prevalence (8.26%, 6.86%, and 5.66% for Locations C, B, and D). Similarly, in samples from Location D, an OTU assigned to the *Lactobacillius* genus was found to have a relative abundance of 36.57% but was found at ≤1% abundance in other locations. Several *Lactobacillus* species have been cited in food aging fermentation processes, including dry aging of beef (Epley, 1992; Hugas and Monfort, 1997; Fadda et al., 2010). The sequence data or database was not sufficient to assign these OTUs to a species, though further culture-based experimentation may be able to elucidate these bacterial taxonomic identities and potential roles during the dry aging process.

Bacterial data: Beta diversity

Along with assessing abundances of specific OTUs in the samples, the study also analyzed the differences between whole communities in each sample. PERMANOVA and plotting of the abundance-weighted Jaccard distances for the 4 locations indicated that the samples originating from the same location were similar to each other while samples differed significantly between locations (PERMANOVA $P < 0.001$). When assessed by other variables including position, section (inner vs. outer), and replicate, samples did not cluster according to these variables as they did by the location (Figure 8A-8C) and were not significantly different between samples from each category (PERMANOVA $P > 0.05$). The PERMDISP analysis revealed that there was heterogeneity of variance within the location variable as well as the section and position variables. While
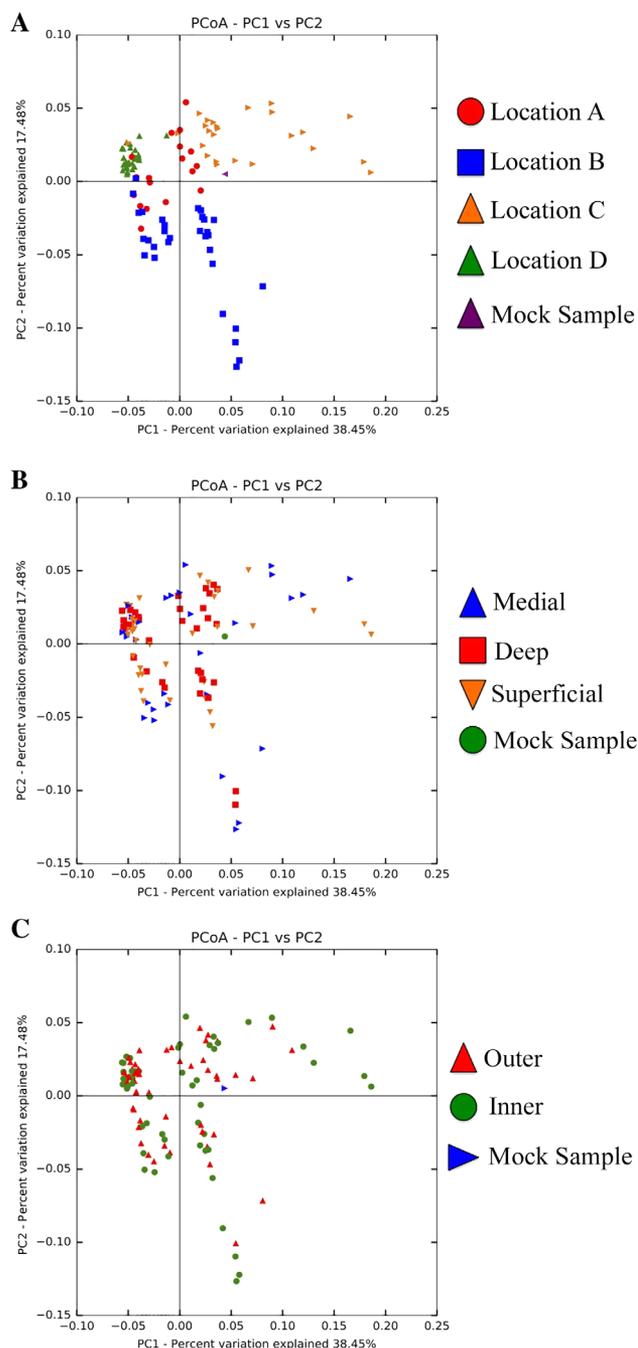
Figure 8. PCoA plots of abundance-weighted Jaccard distances between bacterial communities on dry-aged beef by (A) location, (B) position on loin, and (C) section. While strong clustering of the communities is visible for the location variable, there is no distinct visual separation of the samples when sorted by position on loin or section. PCoA, Principle Coordinate Analysis.

higher levels of dispersion can confound separation of microbial communities, the structures of the communities within each location were similar to one another and indicate that location is the main driver of community composition. No other variables were determined to have significant influence on the diversity and ratios of

the fungal and bacterial OTUs within the samples. Microbial communities are relatively consistent throughout the strip loin and throughout the aging facility, and differences in microbial populations that may influence sensory properties of the meat are mainly products of the aging facility's local microbes and environmental conditions.

Conclusions

There is remarkable diversity in the microflora colonizing beef during the dry aging process, yet a small handful of taxa ultimately dominated the communities observed. These taxa varied notably in their proportions between aging facilities and may be associated with beneficial enzymatic digestion of the tissue contributing to palatability improvements—further research is necessary to confirm these hypotheses. The aging facility —and their unique environmental conditions—appear to be a main source of variation in the microbial communities present on the strip loin, and taxa appear to be distributed consistently on the entire surface of the strip loin and to a depth of 1 cm into the loin. Investigation into the actual mycotoxogenic potential and ways to manage the growth of such fungi, possibly using starter community cultures, would be a prudent subsequent step for producers of dry-aged beef. Additionally, as the analysis was focused on the surface layer of the meat that is removed prior to consumption, further study of how far the physical biomass of these OTUs are found within the loin and how far their exudate penetrates the tissue will help us understand more fully the ways in which the microbes can impact beef in the dry aging process. Understanding the microbiome on the surface of dry-aged meat will lead to a better understanding of which microbes are important, or even necessary, for imparting preferable texture and flavor in the aging process, which will further meat palatability investigations.

Literature Cited

Breuer, U., and H. Harms. 2006. Debaryomyces hansenii—an extremophilic yeast with biotechnological potential. Yeast. 23:415–437. http://doi.org/10.1002/yea.1374.

Brooks F. T., and C. G. Hansford. 1923. Mould growths upon cold-store meat. T. Brit. Mycol. Soc. 8:113–142. https://doi.org/10.1016/S0007-1536(23)80020-1.

Campbell R. E., M. C. Hunt, P. Levis, and E. Chamber IV. 2001. Dry-aging effects on palatability of beef longissimus muscle. J

Food Sci. 66:196–199. https://doi.org/10.1111/j.1365-2621.2001.tb11315.x,

Cook P. E. 1995. Fungal ripened meats and meat products. In: Fermented meats. Springer US, Boston, MA. p. 110–129. https://doi.org/10.1007/978-1-4615-2163-1_5.

Dashdorj D., V. K. Tripathi, S. Cho, Y. Kim, and I. Hwang. 2016. Dry-aging of beef: Review. J. Anim. Sci. Tech. 58:20. https://doi.org/10.1186/s40781-016-0101-9.

DeGeer S. L., M. C. Hunt, C. L. Bratcher, B.A. Crozier-Dodson, D. E. Johnson, and J. F. Stika. 2009. Effects of dry-aging of bone-in and boneless strip loins using two aging processes for two aging times. Meat Sci. 83:768–774. https://doi.org/10.1016/j.meatsci.2009.08.017.

DeSantis T. Z., P. Hugenholtz, N. Larsen, M. Rohas, E. L. Brodie, K. Keller, T. Huber, D. Dalevi, P. Hu, and G. L. Andersen. 2006. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl. Environ. Microbiol. 72:5069–5072. doi: 10.1128/AEM.03006-05.

Edgar R. C., B. J. Haas, J. C. Clemente, C. Quince, and R. Knight. 2011. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics. 27:2194–2200. doi: 10.1093/bioinformatics/btr381.

Epley R. J. 1992. Aging beef. Minnesota Extension Service. University of Minnesota Digital Conservancy. St. Paul, MN. http://hdl.handle.net/11299/51510. (Accessed 12 December 2019).

Ercolini D., A. Casaburi, A. Nasi, I. Ferrocino, R. Di Monaco, P. Ferranti, G. Mauriello, and F. Villani. 2010. Different molecular types of Pseudomonas fragi have the same overall behaviour as meat spoilers. Int. J. Food Microbiol. 142:120–131. https://doi.org/10.1016/j.ijfoodmicro.2010.06.012.

Fadda S., C. López, and G. Vignolo. 2010. Role of lactic acid bacteria during meat conditioning and fermentation: Peptides generated as sensorial and hygienic biomarkers. Meat Sci. 86:66–79. https://doi.org/10.1016/j.meatsci.2010.04.023.

Frisvad, J. C. 1988. Fungal species and their specific production of mycotoxins. In: R. A., Samson and E. S., van Reenen-Hoekstra, editors, Introduction to food-borne fungi. Centraalbureau voor Schimmelcultures, Baarn, Netherlands. p. 239–349

Hebraud M., E. Dubois, P. Potier, and J. Labadie. 1994. Effect of growth temperatures on the protein levels in a psychrotrophic bacterium, Pseudomonas fragi. J Bacteriol. 176:4017–4024. doi: 10.1128/jb.176.13.4017-4024.1994.

Hugas M., and J. M. Monfort. 1997. Bacterial starter cultures for meat fermentation. Food Chem. 59:547–554. https://doi.org/10.1016/S0308-8146(97)00005-8.

Hussong R. V., H. F. Long, and B. W. Hammer. 1937. Classification of the organisms important in dairy products II. Pseudomonas fragi. Iowa Agriculture and Home Economics Experiment Station Research Bulletin. Iowa State University Digital Repository. https://lib.dr.iastate.edu/researchbulletin/vol20/iss225/1/. (Accessed 12 December 2019).

Jeremiah L. E., and L. L. Gibson. 2003. The effects of postmortem product handling and aging time on beef palatability. Food Res. Int. 36:929–941. https://doi.org/10.1016/S0963-9969(03)00102-9.

Jessen B. 1995. Starter cultures for meat fermentations. In: G. Campbell-Platt and P. E. Cook, editors. Fermented meats.

Springer US, Boston, MA. p. 130–159. https://doi.org/10.1007/978-1-4615-2163-1_6.

Khan M. I., S. Jung, K. C. Nam, and C. Jo. 2016. Postmortem aging of beef with a special reference to the dry-aging. Korean J. Food Sci. Anim. Resour. 36:156–169. doi: 10.5851/kosfa.2016.36.2.159.

Kõljalg U., K-H. Larsson, K. Abarenkov, R. H. Nilsson, I. J. Alexander, U. Eberhardt, S. Erland, K. Høiland, R. Kjøller, E. Larsson, T. Pennanen, R. Sen, A. F. S. Taylor, L. Tedersoo, and T. Vrålstad. 2005. UNITE: A database providing web-based methods for the molecular identification of ectomycorrhizal fungi. New Phytol. 166:1063–1068. doi: 10.1111/j.1469-8137.2005.01376.x.

Laster M. A. 2007. Tenderness, flavor, and yield assessment of dry-aged beef. M.S. thesis, Texas A&M University, College Station, TX. (https://www.beefresearch.org/CMDocs/BeefResearch/PE_Project_Summaries/FY06Tenderness_flavor_yield_assessments.pdf).

Lee H. J., J. W. Yoon, M. Kim, H. Oh, Y. Yoon, and C. Jo. 2019. Changes in microbial composition on the crust by different air flow velocities and their effect on sensory properties of dry-aged beef. Meat Sci. 153:152–158. https://doi.org/10.1016/j.meatsci.2019.03.019.

Li Y-F., P-H. Chen, and Z. Yu. 2014. Spatial and temporal variations of microbial community in a mixed plug-flow loop reactor fed with dairy manure. Microb. Biotechnol. 7:332–346. https://doi.org/10.1111/1751-7915.12125.

Nierop Groot M. N., T. Abee, and H. van Bokhorst-van de Veen. 2019. Inactivation of conidia from three Penicillium spp. isolated from fruit juices by conventional and alternative mild preservation technologies and disinfection treatments. Food Microbiol. 81:108–114. https://doi.org/10.1016/j.fm.2018.06.004.

Núñez F., M. Carmen Díaz, M. Rodríguez, E. Aranda, A. Martín, and M. A. Asensio. 2000. Effects of substrate, water activity, and temperature on growth and verrucosidin production by Penicillium polonicum isolated from dry-cured ham. J. Food Protect. 63:231–236. doi:10.4315/0362-028x-63.2.231.

Petersen K. M., S. Westall, and L. Jespersen. 2002. Microbial succession of Debaryomyces hansenii strains during the production of Danish surfaced-ripened cheeses. J Dairy Sci. 85:478–486. https://doi.org/10.3168/jds.S0022-0302(02)74098-8.

Ryu S., M. R. Park, B. E. Maburutse, W. J. Lee, D-J. Park, S. Cho, I. Hwang, S. Oh, and Y. Kim. 2018. Diversity and characteristics of the meat microbiological community on dry-aged beef. Microb. Biotechnol. 28:105–108. https://doi.org/10.4014/jmb.1708.08065.

Savell J. W. 2008. Dry-aging of beef, executive summary. National Cattlemen's Beef Association. https://www.beefresearch.org/cmdocs/beefresearch/dry%20Aging%20of%20beef.pdf. (Accessed 1 March 2020).

Smith D. P., and K. G. Peay. 2014. Sequence depth, not PCR replication, improves ecological inference from next generation DNA sequencing. PLOS ONE. 9(2):e90234. https://doi.org/10.1371/journal.pone.0090234.

Sonjak S., M. Li en, J. C. Frisvad, and N. Gunde-Cimerman. 2011. The mycobiota of three dry-cured meat products from Slovenia. Food Microbiol. 28:373–376. https://doi.org/10.1016/j.fm.2010.09.007.

USDA. 2019. Certified Angus Beef Program G-1 Specification. Agricultural Marketing Service, Washington, DC. June 27, 2019. https://www.ams.usda.gov/sites/default/files/media/G1CertifiedAngusBeef.pdf. (Accessed September 2019).

Van der Walt, J. P., M. T. Smith, and Y. Yamada. 1989. Debaryomyces udenii, sp. nov. (Saccharomycetaceae), a new species from soil. Antonie van Leeuwenhoek. 56:233–239. https://doi.org/10.1007/BF00418935.

Warren K. E., and C. L. Kastner. 1992. A comparison of dry-aged and vacuum-aged beef striploins. J. Muscle Foods. 3:151–157. https://doi.org/10.1111/j.1745-4573.1992.tb00471.x.

Williams A. P. 1990. Penicillium and Aspergillus in the food microbiology laboratory. In: Modern concepts in Penicillium and Aspergillus classification. Springer US, Boston, MA. p. 67–71. https://doi.org/10.1007/978-1-4899-3579-3_6.