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Assessment of microbial ecology in artisanal salami during maturation via metataxonomic analysis

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Abstract

The microbiota in artisanal fermented products plays a crucial role in determining the quality, color, texture, and flavor of salami. Thus, the aim of this study was to identify the microbial population in salami samples throughout the maturation process. Species identification was performed using second-generation high-throughput sequencing of the intergenic ITS region for fungi and the V3/V4 regions of the 16S rRNA gene for bacteria. For bacteria, 197 genera and 572 species were identified during maturation. *Acinetobacter* spp. (13%), *Enterobacter* (10%), *Enterococcus* (9%), and *Bacillus* (9%) were more abundant on day 0. On day 14 of fermentation, the predominant genera were *Acinetobacter* (20%), *Enterobacter* (18%), *Citrobacter* (17%), lactic acid bacteria genera (20%), and *Aeromonas* (10%). At the end of maturation (day 28), *Companilactobacillus* (10%) and *Staphylococcus* (64%) were predominant. In addition, 39 genera and 76 species of fungi were found throughout maturation. The most abundant fungal genera on day 0 were *Yarrowia* (24.96%), *Pichia* (23.91%), *Fusarium* (10.99%), and *Candida* (10.38%). On day 14, the prominent fungal genera were *Hyphopichia* (73.85%) and *Yarrowia* (18.81%), while on the 28th day, *Hyphopichia* (73.73%), *Aspergillus* (14.61%), and *Wallemia* (6.30%) were predominant. Finally, this study was able to identify the total microbiota using a metataxonomic approach.

Keywords: artisanal sausage; fungi; bacteria; metataxonomics.

1 INTRODUCTION

Artisanal animal-derived food products are prepared using raw materials of animal origin, sourced from either self-production or specific origins (Franciosa et al., 2018). The production processes for these products are predominantly manual and subject to inspection controls, which aim to preserve the unique, traditional, cultural, and regional characteristics of the product (Brasil, 2019). An example of such artisanal products widely found in the southern region of Brazil is fermented meat products, such as salami (Schmitt, 2017).

Fermented meat products are made from edible meats or organs and can undergo curing, cooking, smoking, and drying processes before being stuffed into natural or artificial casings (Brasil, 2017). These products involve lactic fermentation of a mixture of meat pieces, fatback, salt, sugar, and spices, which can be intensified by the addition of curing agents, reducers, and starter cultures to ensure better standardization of the final product (Cruxen et al., 2019; Manassi et al., 2022). The production process of fermented sausages includes meat grinding with the addition of fat, salt, curing agents, and seasonings. The interactions of chemical, physical, and microbiological processes during this production phase significantly influence the quality and characteristics of the final product (Gottardo et al., 2011). Fermented meat products are complex microbial ecosystems where bacteria, yeasts, and filamentous fungi coexist. In this environment, microorganisms interact with each other, potentially making the environment more or less favorable for the growth of specific microorganisms. These interactions can modulate changes that occur during fermentation and drying, impacting the aroma, color, and texture of salamis (Franciosa et al., 2018).

The composition of microbiota in fermented meat products varies due to factors such as raw materials, equipment, and fermentation facilities (Roselino & Cavallini, 2016). The diversity of these products results in many unidentified and uncharacterized strains. Understanding the microbiota in artisanal fermented sausages is crucial for developing new starter cultures and ensuring quality standards. In this regard, traditional methods such as plate counting of microorganisms, isolation, and biochemical identification have been used to study the microbial composition of these products. However, only easily cultivable microorganisms can be identified, limiting the detection of those requiring more complex growth conditions (Rantsiou et al., 2005). One possibility for identifying all microorganisms is through the use of molecular methods to detect those present in food,

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stemming from the development of molecular microbiology and the knowledge that DNA carries hereditary information in an encrypted form (Franciosa et al., 2018; Mardanov et al., 2018).

DNA sequencing-based methods are employed because they can be stored in online databases, facilitating data sharing (Cunha, 2016). This sequencing can be performed by analyzing various regions of genes that exhibit high variability, depending on the species, which may require the use of specific markers initially. The 16S rDNA gene is present in all prokaryotes, featuring both conserved and variable regions that evolve at different rates. It is essential for determining phylogenetic relationships and is considered the gold standard for bacterial taxonomy (Cunha, 2016). This methodology enables metataxonomic studies of meat and fermented meat products (Ferrocino et al., 2018; Franciosa et al., 2018).

In this context, metataxonomics stands out by conducting sequencing to identify the entire microbiota of a sample through marker genes such as the 16S gene for bacteria and the ITS regions for fungal identification. These regions are spacers between the 18S, 5.8S, and 28S genes. This methodology provides taxonomic results from the phylum to the species level of the identified microorganisms, using bioinformatics tools and public databases such as GreenGene and RibosomalDatabase (De Cesare, 2019; Franciosa et al., 2018).

In fermented pork sausages, metataxonomic studies enable monitoring the microbiota throughout the fermentation process. This tool allows the identification of strains that contribute to the development of desirable compounds characteristic of artisanal fermented sausages and the identification of potential cultures with ecological interactions that enhance product functionality (Franciosa et al., 2018; Mrkonjic Fuka et al., 2020). Thus, the objective of this study was to evaluate the fungal and bacterial microbiota present in artisanal salamis and to identify competitive or beneficial interactions between fungi and bacteria during the fermentation period using genetic sequencing.

2 MATERIALS AND METHODS

2.1 Salami production

The fermented meat product was prepared using pork meat from an artisanal meat processing producer, which constituted 85% of the raw material. The meat underwent grinding, and ground fatback, which constituted the remaining 15% of the raw material, was added. Subsequently, other ingredients were added to the raw material (meat + fatback) as follows: 2.5% iodized refined salt (Graça Salt Refinery Ltd., Mossoró, Rio Grande do Norte, Brazil), 0.03% black pepper (Valar Food Industry, São Miguel do Oeste, Santa Catarina, Brazil), 0.015% garlic (artisanal producer), 0.12% sugar (Estrela, Passa Tempo Sugar Mill, Rio Brilhante, Mato Grosso do Sul, Brazil), and 5 mL of vinegar per kilogram. The mixture was then homogenized, and the sausage stuffing was performed using dried natural bovine casings with a caliber of 42 (Vita Casings, Getúlio Vargas, Rio Grande do Sul, Brazil), which had been previously sanitized. This process was carried out to obtain samples at 0, 14, and 28 days of maturation, a stage during which the product undergoes fermentation. These steps were performed in triplicate, with each sample weighing approximately 200 g.

Immediately after stuffing, the 0-day maturation samples were packaged and stored at -18°C for subsequent analysis. The remaining samples at 14 and 28 days were smoked for 3 h and then placed in a well-ventilated area for the duration of the maturation period, following the same storage procedure as described for the 0-day samples.

2.2 Metataxonomic analysis

Microbial diversity was studied based on sequenced libraries using the MiSeq Sequencing System (Illumina Inc., USA) and the V2 kit with 300 cycles for single-end sequencing. For sequencing, initially, a 25 g aliquot of the sample was weighed and homogenized with 225 mL of tryptone saline solution. Following this step, DNA extraction was carried out using the magnetic beads technique with a proprietary protocol developed by Neoprospecta Microbiome Technologies, Brazil. For bacteria, amplification was performed using the primers 341F (CCTACGGGRSGCAGCAG) (Y. Wang & Qian, 2009) and 806R (GGACTACHVGGGTWTCTAAT), which are universal for the V3/V4 region of the 16S rRNA gene (Caporaso et al., 2012). For fungi, amplification was generated with primers targeting the ITS1 region, namely, ITS1 (GAACCWGCGGARGGATCA) (White et al., 1990) and ITS2 (GCTGCGTTCTTCATCGATGC) (White et al., 1990). The polymerase chain reaction was performed in triplicate using the Platinum Taq Polymerase (Invitrogen, USA) under the following conditions: 95°C for 5 min, 25 cycles of 95°C for 45 s, 55°C for 30 s, and 72°C for 45 s, followed by a final extension at 72°C for 2 min. Sequences were analyzed using a proprietary pipeline and library preparation protocol (Neoprospecta Microbiome Technologies, Brazil).

2.3 Bioinformatics

For bacteria, sequences were analyzed using a proprietary pipeline (Neoprospecta Microbiome Technologies, Brazil). Each DNA sequence resulting from sequencing passed through an individual quality filter based on the cumulative error probabilities of its bases, allowing a maximum of 1% cumulative error. Subsequently, sequences corresponding to Illumina technology adapters were removed. Sequences that passed the initial procedures and had 100% identity were grouped into phylotypes or clusters and used for taxonomic identification by comparison with a database of accurate 16S rRNA sequences (NeoRef, Neoprospecta Microbiome Technologies, Brazil).

Fungal sequencing data were analyzed using the Sentinel pipeline. Quality assessment of Phred scores (QP) for fastq files in the Sentinel pipeline was performed using FastQC v.0.11.8 (Andrews, n.d.). Subsequently, these files underwent primer trimming and removal of low-quality sequences (Phred < 20) through proprietary Python-based software inspired by the BioPython project (Cock et al., 2009). Clusters with abundances lower than

two were associated with chimeric sequences (Smyth et al., 2010) and were thus excluded from the analyses. Blastn v.2.6.0+ (Altschul et al., 1990) was used to obtain identifications, with a proprietary database as a reference. Species determination was established through a Python-based rule that evaluated whether one of three criteria was met by the hits:

- a higher bit-score;
- a lower e-value;
- taxonomies with greater representation.

The representative species were selected from hits that met one of these criteria. DNA sequences were compared to proprietary or publicly available databases (Quast et al., 2012) and Greengenes (DeSantis et al., 2006), which contain previously characterized DNA sequences.

3 RESULTS AND DISCUSSION

3.1 Bacterial analysis

For bacteria, a total of 335,024 reads were identified, of which only 784 were present on day 0. This quantity was relatively lower than the reads found on days 14 and 28, which amounted to 167,664 and 166,576 reads, respectively. According to Stellato et al. (2016), the complexity of the initial microbiota in fermented meat products is expected due to the intricate microbiome in the salami production environment. The initial composition of the product's microbiota is linked to the materials on the surfaces where the samples were processed after slaughter, the utensils used in processing, and the microbiota present in the air and on the surface of the meat pieces (Stellato et al., 2016).

Regarding the abundance of bacterial genera (Figure 1) in the salami sample at day 0, it was observed that the most abundant genera were *Acinetobacter* spp. (13.14%), *Enterobacter* spp. (10.07%), *Enterococcus* spp. (8.80%), and *Bacillus* spp. (8.55%). In the following sampling, after the start of the fermentation process (day 14), there was an approximately 114% increase in the number of reads. The genera *Acinetobacter* spp. (19.95%), *Enterobacter* spp. (17.62%), *Citrobacter* spp. (17.39%), *Aeromonas* spp. (9.53%), and *Lactobacillus* spp. (7.99%) were the most abundant on day 14. At the end of maturation (day 28), the read count decreased by only 0.65% compared to day 14, demonstrating a certain stability in the bacterial ecosystem. The relative abundances for the genera were *Staphylococcus* spp. (63.58%), *Companilactobacillus* spp. (9.92%), and *Citrobacter* spp. (6.65%).

Through the analysis of bacteria present in the salami, over 570 species were identified (Table 1). Out of these, 24 species were highlighted (Figure 2). The most abundant on day 0 were

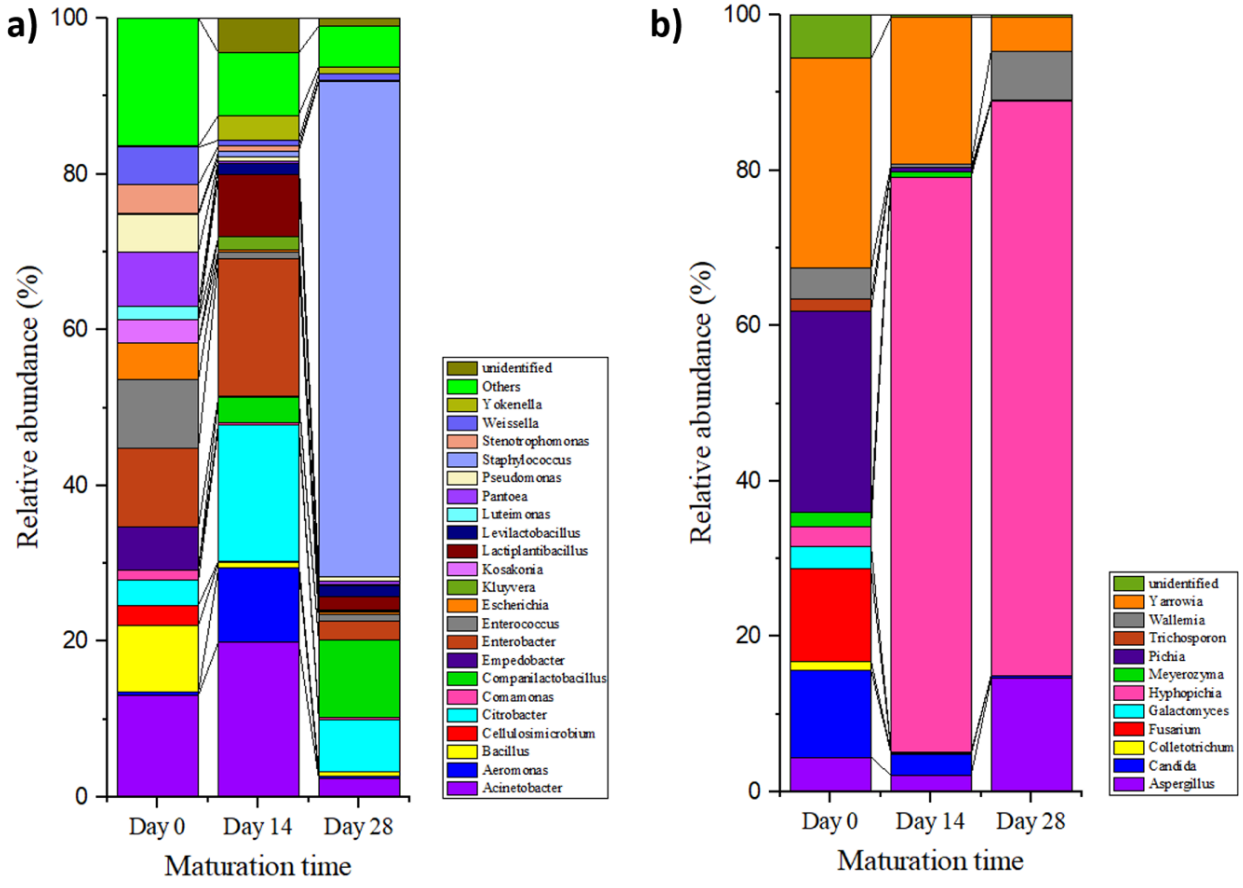

Figure 1. Relative abundance (%) of (a) bacterial and (b) fungal genera on days 0, 14, and 28 of artisanal salami maturation.

Table 1. Number of reads of bacterial species identified throughout the maturation period of artisanal salami.

Species	Total	Day 0	Day 14	Day 28
Acetobacter indonesiensis	37	0	15	22
Acetobacter lambici	9	0	6	3
Acetobacter orientalis	32	3	9	20
Acetobacter pasteurianus	11	0	7	4
Acetobacter persici	13	0	4	9
Acetobacter tropicalis	16	0	12	4
Acholeplasma axanthum	2	0	2	0
Achromobacter denitrificans	3	0	0	3
Achromobacter piechaudii	12	0	1	11
Achromobacter xylosoxidans	38	0	16	22
Acidovorax wautersii	8	0	2	6
Acinetobacter baumannii	2,028	46	831	1,151
Acinetobacter beijerinckii	146	0	135	11
Acinetobacter bereziniae	5,897	7	5,444	446
Acinetobacter bouvetii	44	0	42	2
Acinetobacter calcoaceticus	441	23	177	241
Acinetobacter genomosp. C1	6	0	6	0
Acinetobacter gerneri	31	0	2	29
Acinetobacter guillouiae	21	0	10	11
Acinetobacter gyllenbergii	828	1	783	44
Acinetobacter haemolyticus	3	0	3	0
Acinetobacter indicus	151	1	59	91
Acinetobacter johnsonii	4,503	5	4,251	247
Acinetobacter junii	24	0	23	1
Acinetobacter lwoffii	13	0	3	10
Acinetobacter nosocomialis	29	0	15	14
Acinetobacter oleivorans	35	0	16	19
Acinetobacter parvus	397	0	376	21
Acinetobacter schindleri	28	0	7	21
Acinetobacter soli	21	0	7	14
Acinetobacter sp.	704	0	646	58
Acinetobacter tandoii	13,997	9	13,743	245
Acinetobacter tjernbergiae	1,451	0	1,273	178
Acinetobacter ursingii	390	11	231	148
Acinetobacter venetianus	6,398	0	5,371	1,027
Actinoplanes couchii	25	0	0	25
Aeribacillus pallidus	50	0	16	34
Aerococcus viridans	83	0	53	30
Aeromicrobium alkaliterrae	9	0	4	5
Aeromicrobium erythreum	6	0	0	6
Aeromicrobium ginsengisoli	14	0	10	4
Aeromicrobium halocynthiae	6	0	0	6
Aeromicrobium massiliense	4	0	4	0
Aeromicrobium tamlense	6	0	0	6
Aeromonas caviae	1,013	0	861	152
Aeromonas dhakensis	9,557	0	9,515	42
Aeromonas eucrenophila	3	0	3	0
Aeromonas hydrophila	2,645	0	2,579	66
Aeromonas media	8	0	5	3
Aeromonas molluscorum	42	0	33	9
Aeromonas sanarellii	4	0	4	0
Aeromonas veronii	3,099	3	2,981	115
Agaricicola taiwanensis	2	0	2	0
Agrobacterium larrymoorei	25	2	15	8
Agrobacterium tumefaciens	111	0	66	45
Alcaligenes faecalis	7	0	2	5
Alcanivorax pacificus	24	0	15	9
Algoriphagus terrigena	2	0	2	0
Aliihoeflea aestuarii	62	0	32	30
Alishewanella aestuarii	3	0	0	3
Alkaliphilus crotonatoxidans	5	0	0	5
Alkaliphilus oremlandii	4	0	4	0
Amphibacillus sediminis	4	0	4	0
Aquabacter spiritensis	9	0	2	7
Aquitalea magnusonii	1,518	0	1,131	387
Arcobacter butzleri	44	0	17	27
Arcobacter cryaerophilus	4	0	4	0
Arthrobacter gandavensis	11	0	1	10
Arthrobacter mysorens	2	0	1	1
Arthrobacter nicotianae	2	0	0	2
Arthrobacter oxydans	2	0	2	0
Arthrobacter protophormiae	17	0	9	8
Arthrobacter woluwensis	2	0	2	0
Aureimonas altamirensis	72	3	25	44
Aureimonas frigidaquae	9	0	1	8
Aureimonas jatrophae	3	0	0	3
Aureimonas phyllosphaerae	4	0	2	2
Bacillus acidicola	3	0	1	2
Bacillus carboniphilus	10	0	0	10
Bacillus cecembensis	5	0	0	5
Bacillus cereus sp. group	16	0	9	7
Bacillus circulans	55	0	33	22
Bacillus clausii	573	34	285	254
Bacillus coagulans	28	0	18	10
Bacillus firmus	19	0	4	15
Bacillus flexus	27	0	6	21
Bacillus gibsonii	201	0	116	85
Bacillus ginsengihumi	2	0	0	2
Bacillus megaterium	43	0	34	9
Bacillus nealsonii	10	0	3	7
Bacillus niacini	2	0	2	0
Bacillus oshimensis	11	0	6	5
Bacillus pseudalcaliphilus	2	0	0	2
Bacillus pumilus	246	0	128	118
Bacillus shackletonii	2	0	0	2
Bacillus siralis	33	0	21	12
Bacillus subtilis group	840	14	453	373
Bacillus thermoamylovorans	82	19	39	24
Bacillus thermolactis	24	0	10	14
Bacillus thermozeamaize	4	0	4	0
Bacillus trypoxylicola	7	0	0	7
Bacteroides faecis	3	0	0	3
Bacteroides graminisolvens	2	0	0	2
Bacteroides ovatus	2	0	2	0
Bavariicoccus seileri	3	0	2	1
Bordetella avium	34	0	17	17

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Table 1. Continuation.

Species	Total	Day 0	Day 14	Day 28
Bordetella petrii	20	0	13	7
Bosea minatitlanensis	3	0	3	0
Bosea thiooxidans	9	0	1	8
Brachybacterium arcticum	3	0	3	0
Brachybacterium faecium	301	0	147	154
Brachybacterium muris	2	0	2	0
Brachybacterium nesterenkovii	26	0	20	6
Brachybacterium paraconglomeratum	12	0	0	12
Brachybacterium sacelli	7	0	7	0
Bradyrhizobium elkanii	3	0	0	3
Brevibacillus invocatus	5	0	5	0
Brevibacillus limnophilus	8	0	8	0
Brevibacterium epidermidis	27	0	20	7
Brevibacterium linens	4	0	0	4
Brevibacterium oceani	15	0	2	13
Brevibacterium salitolerans	20	0	9	11
Brevibacterium senegalense	90	0	34	56
Brevundimonas abyssalis	2	0	2	0
Brevundimonas aurantiaca	8	0	4	4
Brevundimonas bacteroides	8	0	8	0
Brevundimonas diminuta	20	0	14	6
Brevundimonas faecalis	5	0	0	5
Brevundimonas terrae	113	0	57	56
Brevundimonas vesicularis	10	0	4	6
Caldibacillus debilis	2	0	2	0
Candidatus Devosia euplotis	7	0	0	7
Castellaniella denitrificans	7	0	6	1
Cellulomonas cellasea	4	0	2	2
Cellulomonas denverensis	76	0	21	55
Cellulomonas flavigena	12	0	6	6
Cellulomonas hominis	77	0	33	44
Cellulomonas septica	2	0	1	1
Cellulosimicrobium cellulans	378	18	236	124
Cellulosimicrobium funkei	3	0	2	1
Cellulosimicrobium terreum	32	2	12	18
Chelatococcus daeguensis	9	0	8	1
Chryseobacterium culicis	12	0	4	8
Chryseobacterium hagamense	2	0	0	2
Chryseobacterium hominis	13	0	4	9
Chryseobacterium indoltheticum	12	0	5	7
Chryseobacterium taeanense	3	0	3	0
Chryseobacterium taichungense	4	0	4	0
Chryseobacterium taiwanense	3	0	3	0
Chryseobacterium vrystaatense	3	0	0	3
Citrobacter amalonaticus	10	0	3	7
Citrobacter braakii	43	0	28	15
Citrobacter freundii	34,775	21	25,391	9,363
Citrobacter koseri	20	0	17	3
Citrobacter murliniae	2,558	4	1,898	656
Citrobacter rodentium	37	0	32	5
Citrobacter sedlakii	10	0	8	2
Citrobacter werkmanii	2,813	0	1,790	1,023

Continue...

Table 1. Continuation.

Species	Total	Day 0	Day 14	Day 28
Citrobacter youngae	4	0	1	3
Clavibacter michiganensis	3	0	1	2
Clostridium baratii	2	0	2	0
Clostridium disporicum	14	0	4	10
Clostridium intestinale	5	0	4	1
Clostridium sp.	2	0	2	0
Comamonas aquatica	178	0	68	110
Comamonas kerstersii	496	11	230	255
Comamonas terrigena	12	0	8	4
Comamonas testosteroni	444	0	387	57
Corynebacterium acetoacidophilum	5	0	5	0
Corynebacterium casei	30	0	14	16
Corynebacterium deserti	23	0	18	5
Corynebacterium freneyi	3	0	0	3
Corynebacterium glutamicum	71	0	35	36
Corynebacterium simulans	7	0	7	0
Corynebacterium stationis	18	0	11	7
Corynebacterium terpenotabidum	4	0	0	4
Corynebacterium variabile	11	0	5	6
Cronobacter dublinensis	110	2	30	78
Cronobacter helveticus	2	0	1	1
Cronobacter pulveris	2	0	2	0
Cronobacter sakazakii	126	0	99	27
Cronobacter turicensis	7	0	3	4
Curtobacterium citreum	22	0	6	16
Curtobacterium flaccumfaciens	26	0	17	9
Delftia acidovorans	2	0	2	0
Delftia tsuruhatensis	4	0	4	0
Desemzia incerta	2	0	0	2
Devosia albogilva	4	0	2	2
Devosia chinhatensis	7	0	7	0
Devosia hwasunensis	2	0	2	0
Devosia riboflavina	22	0	18	4
Diaphorobacter nitroreducens	2	0	0	2
Dickeya chrysanthemi	11	0	0	11
Dietzia maris	22	0	9	13
Dysgonomonas capnocytophagoides	52	8	7	37
Dysgonomonas oryzarvi	5	0	3	2
Empedobacter brevis	47	0	42	5
Empedobacter falsenii	385	43	203	139
Ensifer adhaerens	22	0	12	10
Enteric Group 137	2	0	0	2
Enterobacter aerogenes	18,723	14	17,411	1,298
Enterobacter asburiae	28	0	17	11
Enterobacter cancerogenus	12	0	4	8
Enterobacter cloacae	14,512	42	11,916	2,554
Enterobacter hormaechei	380	20	166	194
Enterobacter kobei	14	0	6	8
Enterobacter ludwigii	60	3	26	31
Enterobacteriaceae bacterium	9,248	0	7,531	1,717
Enterococcus asini	2	0	2	0
Enterococcus avium	2	0	1	1
Enterococcus casseliflavus	1,347	57	615	675

Continue...

Table 1. Continuation.

Species	Total	Day 0	Day 14	Day 28
Enterococcus columbae	8	0	6	2
Enterococcus devriesei	4	0	0	4
Enterococcus faecalis	67	0	36	31
Enterococcus faecium	7	0	0	7
Enterococcus gallinarum	24	0	15	9
Enterococcus gilvus	4	1	2	1
Enterococcus italicus	83	11	26	46
Enterococcus malodoratus	1,305	0	679	626
Enterococcus raffinosus	5	0	4	1
Enterococcus saccharolyticus	10	0	2	8
Enterococcus termitis	6	0	6	0
Erwinia aroideae	15	0	4	11
Erwinia billingiae	3	0	3	0
Erwinia tasmaniensis	2	0	0	2
Erythrobacter gangjinensis	6	0	0	6
Escherichia coli	39	29	4	6
Escherichia hermannii	642	8	430	204
Escherichia sp. KTE31	4	0	3	1
Escherichia vulneris	67	0	37	30
Facklamia tabacinasalis	7	0	0	7
Falsirhodobacter halotolerans	15	0	6	9
Flavobacterium ceti	3	0	2	1
Flavobacterium marinum	2	0	2	0
Flavobacterium ummariense	13	0	3	10
Frateuria aurantia	9	0	3	6
Georgenia satyanarayanai	73	0	26	47
Gluconobacter albidus	2	0	2	0
Gluconobacter cerinus	3	0	0	3
Gluconobacter frateurii	83	0	46	37
Glycomyces mongolensis	5	0	0	5
Gordonia terrae	17	0	6	11
Gracilibacillus dipsosauri	7	0	2	5
Gracilibacillus ureilyticus	2	0	2	0
Grimontella senegalensis	6	0	5	1
Gulosibacter chungangensis	4	0	4	0
Halomonas meridiana	4	0	4	0
Halomonas venusta	4	0	0	4
Halomonas zhanjiangensis	2	0	0	2
Haloquadratum walsbyi	18	0	1	17
Halorubrum orientale	2	0	0	2
Halotalea alkalilenta	40	0	13	27
Herbiconiux ginsengi	2	0	2	0
Hoeflea halophila	3	0	3	0
Hyphomonas polymorpha	4	0	1	3
Isoptericola variabilis	4	0	3	1
Jeotgalicoccus huakuii	2	0	2	0
Kaistobacter terrae	2	0	0	2
Ketogulonicigenium vulgare	127	0	48	79
Klebsiella oxytoca	122	0	81	41
Klebsiella pneumoniae	1,241	5	563	673
Kluyvera ascorbata	2,789	0	2,507	282
Kluyvera cryocrescens	14	0	5	9
Kluyvera georgiana	11	0	3	8

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Table 1. Continuation.

Species	Total	Day 0	Day 14	Day 28
Kluyvera intermedia	363	0	338	25
Kocuria halotolerans	8	0	0	8
Kocuria marina	2	0	2	0
Kosakonia cowanii	191	23	55	113
Kosakonia oryzae	3	0	1	2
Kosakonia radicincitans	5	0	1	4
Kosakonia sacchari	73	0	29	44
Kurthia gibsonii	23	0	15	8
Lactobacillus acidipiscis	1,547	0	423	1,124
Lactobacillus agilis	24	0	9	15
Lactobacillus brevis	3,323	0	1,475	1,848
Lactobacillus casei	2	0	2	0
Lactobacillus curvatus	360	0	40	320
Lactobacillus farciminis	29,298	0	13,118	16,180
Lactobacillus fermentum	33	0	25	8
Lactobacillus futsaii	68	0	35	33
Lactobacillus ghanensis	11	0	1	10
Lactobacillus johnsonii	2	0	2	0
Lactobacillus kimchiensis	13	0	5	8
Lactobacillus koreensis	21	0	0	21
Lactobacillus mali	8	0	4	4
Lactobacillus mindensis	17	0	8	9
Lactobacillus namurensis	854	0	575	279
Lactobacillus nantensis	76	0	28	48
Lactobacillus odoratitofui	3	0	2	1
Lactobacillus parabrevis	275	0	188	87
Lactobacillus paralimentarius	116	0	114	2
Lactobacillus paucivorans	12	0	0	12
Lactobacillus pentosus	4	0	3	1
Lactobacillus plantarum	5,788	0	4,276	1,512
Lactobacillus sakei	5	0	0	5
Lactobacillus salivarius	32	0	10	22
Lactobacillus senmaizukei	12	0	11	1
Lactobacillus spicheri	49	0	47	2
Lactobacillus uvarum	9	0	3	6
Lactobacillus vaccinostercus	4	0	0	4
Lactobacillus versmoldensis	336	0	99	237
Lactobacillus xiangfangensis	4,720	0	3,140	1,580
Lactococcus garvieae	459	0	55	404
Lactococcus lactis	1,052	0	217	835
Lampropedia hyalina	7	0	1	6
Leclercia adecarboxylata	30	0	26	4
Lelliottia amnigena	34	0	14	20
Leucobacter alluvii	4	0	4	0
Leucobacter aridicollis	4	0	2	2
Leucobacter celer	2	0	2	0
Leucobacter chironomi	16	0	11	5
Leucobacter komagatae	19	0	7	12
Leucobacter tardus	98	37	28	33
Leuconostoc citreum	660	0	321	339
Leuconostoc mesenteroides	3	0	3	0
Leuconostoc pseudomesenteroides	103	3	45	55
Luteibacter rhizovicinus	6	0	0	6

Continue...

Table 1. Continuation.

Species	Total	Day 0	Day 14	Day 28
Luteimonas aestuarii	171	14	43	114
Luteimonas composti	5	0	4	1
Luteimonas huabeiensis	181	0	97	84
Luteimonas marina	3	0	3	0
Lysinibacillus fusiformis	73	0	36	37
Lysinibacillus massiliensis	15	0	2	13
Lysinibacillus meyeri	2	0	0	2
Lysinibacillus sphaericus	3	0	0	3
Lysobacter xinjiangensis	4	0	0	4
Macrococcus caseolyticus	3	0	3	0
Mangrovibacter plantisponsor	3	0	3	0
Marinilactibacillus piezotolerans	5	0	1	4
Massilia timonae	3	0	3	0
Mesorhizobium mediterraneum	5	0	5	0
Mesorhizobium thiogangeticum	2	0	2	0
Methylobacillus arboreus	26	0	9	17
Methylobacillus flagellatus	31	0	12	19
Methylobacterium extorquens	7	0	7	0
Methylobacterium komagatae	6	0	6	0
Methylobacterium radiotolerans	9	0	4	5
Microbacterium aurum	4	0	2	2
Microbacterium pumilum	5	0	0	5
Micrococcus luteus	2	0	1	1
Micrococcus xinjiangensis	19	0	11	8
Microvirga aerophila	2	0	2	0
Moraxella osloensis	113	0	103	10
Morganella morganii	1,693	0	1,109	584
Mycetocola lacteus	22	0	8	14
Mycobacterium sp.	3	0	0	3
Mycoplana dimorpha	2	0	2	0
Mycoplana ramosa	10	0	4	6
Myroides marinus	161	0	156	5
Myroides odoratus	2	0	2	0
Myxococcus xanthus	4	0	1	3
Natronomonas gomsonensis	2	0	0	2
Nesterenkonia flava	250	0	125	125
Nesterenkonia halotolerans	31	0	20	11
Nesterenkonia lacusekhoensis	155	0	60	95
Nitratireductor lucknowense	56	0	27	29
Nocardioides dubius	4	0	3	1
Nocardioides mesophilus	2	0	0	2
Nocardiopsis alba	12	0	5	7
Nocardiopsis flavescens	6	0	0	6
Nocardiopsis metallicus	2	0	2	0
Nocardiopsis prasina	5	0	2	3
Nocardiopsis salina	3	0	1	2
Novosphingobium panipatense	63	0	14	49
Novosphingobium resinovorum	35	0	21	14
Oceanobacillus cibarius	2	0	2	0
Oceanobacillus iheyensis	8	0	5	3
Oceanobacillus indicireducens	2	0	2	0
Oceanobacillus oncorhynchi	27	0	12	15
Ochrobactrum anthropi	2	0	2	0

Continue...

Table 1. Continuation.

Species	Total	Day 0	Day 14	Day 28
Ochrobactrum gallinifaecis	7	0	5	2
Ochrobactrum intermedium	61	0	26	35
Ochrobactrum pseudintermedium	24	0	11	13
Ochrobactrum pseudogrignonense	43	0	26	17
Oerskovia ginkgo	12	0	0	12
Olivibacter jilunii	15	0	5	10
Ornithinimicrobium pekingense	16	0	7	9
Oxalicibacterium faecigallinarum	4	0	4	0
Paenibacillus barcinonensis	3	0	1	2
Paenibacillus barengoltzii	4	0	3	1
Paenibacillus camelliae	3	0	3	0
Paenibacillus campinasensis	92	0	14	78
Paenibacillus ginsengihumi	27	0	9	18
Paenibacillus graminis	4	0	0	4
Paenibacillus hunanensis	20	0	8	12
Paenibacillus illinoisensis	29	0	20	9
Paenibacillus massiliensis	5	0	5	0
Paenibacillus montaniterrae	19	0	10	9
Paenibacillus nanensis	6	0	0	6
Paenibacillus pabuli	14	0	13	1
Paenibacillus phoenicis	9	0	3	6
Paenibacillus senegalensis	2	0	1	1
Paenibacillus taohuashanense	13	0	3	10
Paenibacillus turicensis	14	0	6	8
Pantoea agglomerans	323	3	148	172
Pantoea ananatis	107	13	27	67
Pantoea calida	24	0	9	15
Pantoea cedenensis	3	0	0	3
Pantoea dispersa	618	39	199	380
Pantoea septica	36	0	17	19
Pantoea stewartii	28	0	8	20
Pantoea wallisii	7	0	3	4
Paracoccus aestuarii	5	0	0	5
Paracoccus alcaliphilus	52	0	19	33
Paracoccus aminophilus	3	0	0	3
Paracoccus aminovorans	15	0	4	11
Paracoccus chinensis	3	0	0	3
Paracoccus denitrificans	13	0	13	0
Paracoccus kocurii	2	0	0	2
Paracoccus kondratievae	10	0	7	3
Paracoccus solventivorans	14	0	2	12
Paracoccus sphaerophysae	7	0	4	3
Paracoccus yeei	31	0	12	19
Parapedobacter luteus	10	0	3	7
Paucisalibacillus globulus	49	0	19	30
Pectobacterium carotovorum	34	10	4	20
Pediococcus acidilactici	2	0	2	0
Pediococcus pentosaceus	4	0	4	0
Pelagibacterium halotolerans	3	0	3	0
Pelagibacterium luteolum	10	0	3	7
Pigmentiphaga daeguensis	17	0	10	7
Piscicoccus intestinalis	6	0	6	0
Prauserella rugosa	21	0	6	15

Continue...

Table 1. Continuation.

Species	Total	Day 0	Day 14	Day 28
Proteus mirabilis	40	0	38	2
Proteus penneri	59	0	56	3
Proteus vulgaris	522	0	497	25
Providencia alcalifaciens	840	0	840	0
Providencia rettgeri	11	0	3	8
Providencia stuartii	31	0	13	18
Pseudaminobacter salicylatoxidans	13	0	1	12
Pseudochrobactrum saccharolyticum	18	0	13	5
Pseudoclavibacter faecalis	17	0	11	6
Pseudofulvimonas gallinarii	14	0	7	7
Pseudomonas aeruginosa	14	0	4	10
Pseudomonas composti	34	3	12	19
Pseudomonas denitrificans	27	0	10	17
Pseudomonas formosensis	164	0	102	62
Pseudomonas fulva	247	18	86	143
Pseudomonas hibiscicola	7	0	2	5
Pseudomonas indoloxydans	4	0	0	4
Pseudomonas japonica	14	0	14	0
Pseudomonas koreensis	2	0	2	0
Pseudomonas mendocina	69	0	63	6
Pseudomonas nitroreducens	51	0	27	24
Pseudomonas oryzihabitans	25	0	13	12
Pseudomonas plecoglossicida	18	0	12	6
Pseudomonas pseudoalcaligenes	50	8	38	4
Pseudomonas psychrotolerans	11	0	0	11
Pseudomonas putida	908	2	390	516
Pseudomonas stutzeri	152	0	81	71
Pseudomonas taiwanensis	4	0	2	2
Pseudomonas thermotolerans	36	0	30	6
Pseudomonas xanthomarina	9	0	9	0
Pseudomonas xiamenensis	35	0	6	29
Pseudonocardia alni	2	0	1	1
Pseudonocardia ammonioxydans	14	0	5	9
Pseudoxanthomonas suwonensis	128	0	45	83
Pseudoxanthomonas taiwanensis	12	0	9	3
Psychrobacillus psychrotolerans	9	0	2	7
Psychrobacter alimentarius	3	0	0	3
Psychrobacter celer	47	0	34	13
Psychrobacter marincola	4	0	1	3
Psychrobacter sanguinis	28	0	28	0
Pusillimonas noertemannii	38	0	21	17
Raoultella ornithinolytica	17	0	5	12
Raoultella planticola	2	0	2	0
Raoultella terrigena	1,072	0	1,036	36
Rheinheimera perlucida	6	0	2	4
Rhizobium aggregatum	2	0	0	2
Rhizobium cnuense	2	0	0	2
Rhizobium nepotum	9	0	1	8
Rhizobium sp.	21	0	0	21
Rhodococcus fascians	2	0	2	0
Rhodococcus phenolicus	41	27	0	14
Rhodococcus pyridinivorans	26	0	15	11
Rhodococcus rhodochrous	7	0	1	6

Continue...

Table 1. Continuation.

Species	Total	Day 0	Day 14	Day 28
Rhodopseudomonas palustris	5	0	0	5
Rosenbergiella nectarea	10	0	4	6
Roseomonas aerophila	26	0	15	11
Roseomonas aestuarii	4	0	0	4
Roseomonas cervicalis	20	0	16	4
Roseomonas musae	16	0	15	1
Saccharibacillus sacchari	6	0	6	0
Saccharomonospora azurea	6	0	4	2
Saccharomonospora glauca	7	0	3	4
Saccharopolyspora rectivirgula	31	0	17	14
Salinibacterium xinjiangense	2	0	0	2
Salinicoccus kunmingensis	2	0	2	0
Salmonella bongori	566	0	509	57
Sanguibacter soli	13	0	13	0
Sediminihabitans luteus	14	14	0	0
Serratia marcescens	4	0	4	0
Shigella flexneri	6	0	3	3
Solibacillus silvestris	16	0	13	3
Soonwooa buanensis	99	0	97	2
Sphingobacterium bambusae	37	0	19	18
Sphingobacterium composti, Yoo et al. 2007	470	4	208	258
Sphingobacterium detergens	13	0	0	13
Sphingobacterium hotanense	2	0	2	0
Sphingobacterium mizutaii	2	0	2	0
Sphingobacterium multivorum	32	0	32	0
Sphingobacterium thalpophilum	56	0	17	39
Sphingobacterium thermophilum	104	0	49	55
Sphingobacterium wenxiniae	36	0	17	19
Sphingobium lactosutens	2	0	2	0
Sphingobium limneticum	5	0	1	4
Sphingobium yanoikuyae	35	1	13	21
Sphingomonas azotofornans	9	0	0	9
Sphingomonas faeni	3	0	3	0
Sphingomonas hunanensis	14	0	0	14
Sphingomonas koreensis	4	0	3	1
Sphingomonas leidyi	6	0	3	3
Sphingomonas melonis	2	0	2	0
Sphingomonas panni	2	0	2	0
Sphingomonas paucimobilis	2	0	2	0
Sphingomonas roseiflava	2	0	0	2
Sphingomonas sp.	5	0	0	5
Staphylococcus aureus	4	0	4	0
Staphylococcus cohnii	296	0	5	291
Staphylococcus epidermidis	155	0	79	76
Staphylococcus equorum	47	0	0	47
Staphylococcus gallinarum	131	0	75	56
Staphylococcus hominis	33	0	1	32
Staphylococcus kloosii	4	0	4	0
Staphylococcus pasteuri	2	0	0	2
Staphylococcus saprophyticus	103,657	1	68	103,588
Staphylococcus sciuri	181	0	92	89
Staphylococcus warneri	2,525	0	807	1,718

Continue...

Table 1. Continuation.

Species	Total	Day 0	Day 14	Day 28
Staphylococcus xylosus	2	0	1	1
Stenotrophomonas acidaminiphila	2	0	0	2
Stenotrophomonas chelatiphaga	2	0	2	0
Stenotrophomonas maltophilia	1,371	30	1,142	199
Stenotrophomonas rhizophila	13	0	5	8
Streptococcus macedonicus	23	0	15	8
Streptomyces carpaticus	3	0	3	0
Streptomyces diastaticus	7	0	0	7
Streptomyces malachitofuscus	2	0	0	2
Streptomyces phaeopurpureus	3	0	0	3
Streptomyces sodiiphilus	10	0	4	6
Streptomyces sulphureus	2	0	2	0
Symbiobacterium thermophilum	12	0	1	11
Tatumella morbirosei	26	0	20	6
Tatumella punctata	3	0	1	2
Tatumella saanichensis	4	0	4	0
Tepidimicrobium xylanilyticum	4	0	0	4
Terribacillus halophilus	90	0	46	44
Terribacillus saccharophilus	18	0	5	13
Thermoactinomyces intermedius	9	0	0	9
Thermoactinomyces vulgaris	3	0	3	0
Thermobacillus composti	9	0	0	9
Thermomonas brevis	9	0	0	9

Continue...

Table 1. Continuation.

Species	Total	Day 0	Day 14	Day 28
Thermovum composti	11	0	11	0
Trabulsiella odontotermitis	57	0	48	9
Turicibacter sanguinis	2	0	0	2
Ureibacillus suwonensis	34	10	12	12
Ureibacillus thermosphaericus	138	0	40	98
Vagococcus fluvialis	739	0	711	28
Weissella beninensis	5	0	0	5
Weissella confusa	545	21	282	242
Weissella hellenica	8	0	5	3
Weissella paramesenteroides	446	16	270	160
Weissella thailandensis	1,705	0	782	923
Xanthomonas axonopodis	2	0	2	0
Xanthomonas codiaei	5	0	5	0
Xanthomonas translucens	16	0	12	4
Xylanibacterium ulmi	6	0	0	6
Yokenella regensburgei	6,835	1	5,308	1,526
[Cellvibrio] gilvus	2	0	0	2
[Clostridium] saccharolyticum	7	0	4	3
[Clostridium] xylanolyticum	13	0	2	11
[Eubacterium] tenue	2	0	2	0
[Flavobacterium] lutescens	6	6	0	0
Uncultured *Oscillatoria* sp.	73	0	32	41
Total for the sample	335,024	784	167,664	166,576

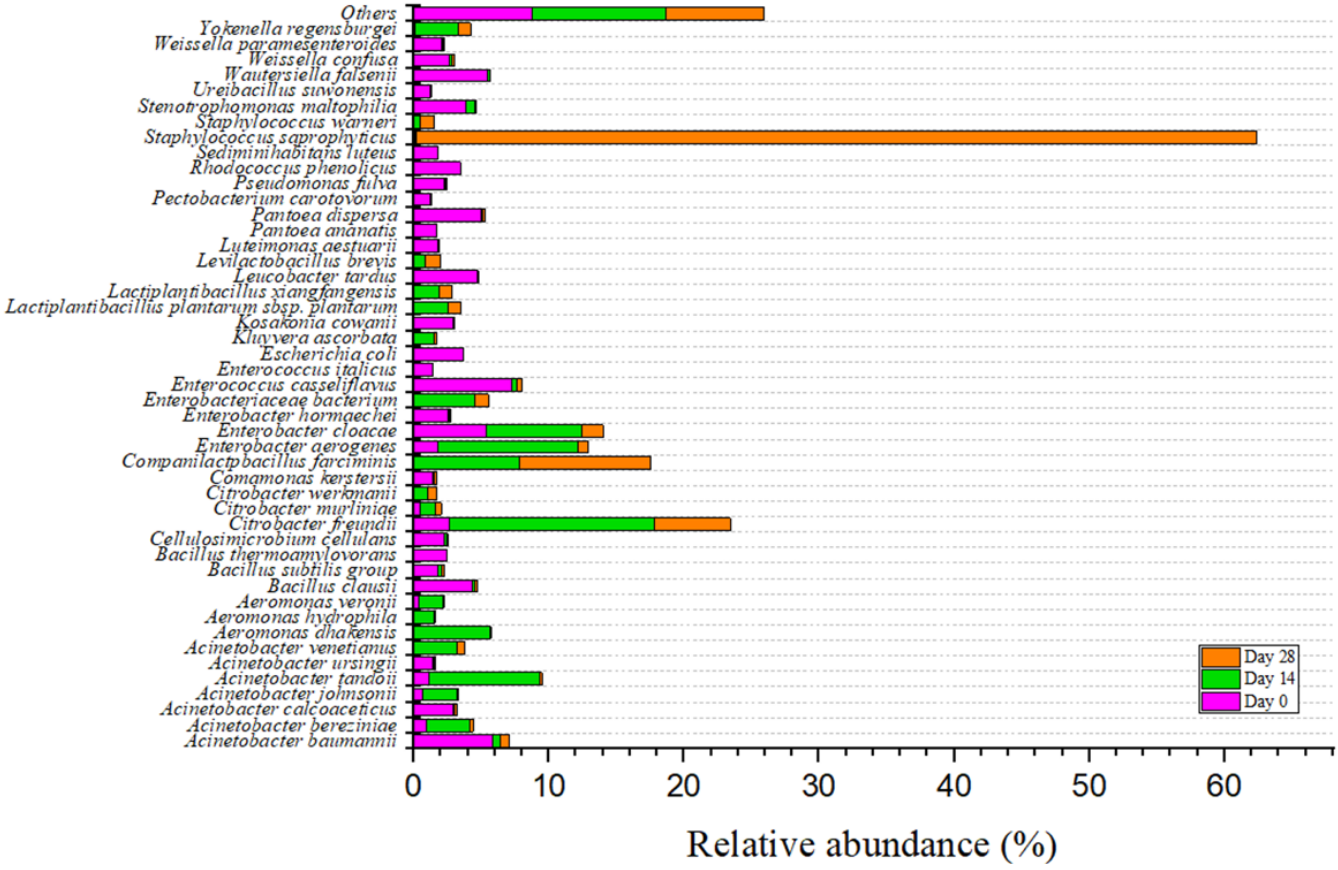

Figure 2. Relative abundance (%) of the most abundant bacterial species on days 0, 14, and 28 of artisanal salami maturation

Enterococcus casseliflavus (7.57%) and *Acinetobacter baumannii* (6.11%). The genera *Enterococcus* spp. and *Acinetobacter* spp. are known to be present in high concentrations in the native microflora of raw meats (Guerrero-Legarreta, 2014), justifying their presence in the salami before maturation. The higher abundance of the genus *Acinetobacter* spp. in the salami at the beginning of the process and on day 14 can be attributed to this genus's capability to utilize various carbon sources for growth, such as hydrocarbons, alcohols, amino acids, aliphatic acids, pentose sugars, and aromatic components. Additionally, it can thrive under different pH, temperature, and high humidity conditions (Chagas, 2015).

The species *E. casseliflavus*, reported by Gomes et al. (2013) in chicken meat, milk, and dairy products, acts as a lactic acid bacterium (LAB) and exhibits inhibitory effects on pathogenic microorganisms like *Staphylococcus aureus* and *Listeria monocytogenes*, which were not detected in the artisanal salami at any point during the maturation period. Therefore, the presence of *E. casseliflavus* in the salami at day 0 likely contributes to inhibiting the growth of certain pathogens, enhancing the safety of the final product. The presence of *A. baumannii* may be attributed to the animal's handling conditions, slaughter methods, processing, and food packaging (Carvalheira et al., 2017).

Still at the beginning of the production process (day 0), *Wautersiella falsenii* (5.71%) emerged as the third most abundant species (Figure 2). The presence of *W. falsenii* before maturation may be correlated with wooden and plastic cutting boards used in salami processing (Abdul-Mutalib et al., 2015). This bacterial species has the potential for growth throughout the salami maturation period since it ferments glucose, a substrate present in the salami formulation (Collins et al., 2018; Zaman et al., 2017; Zeng et al., 2020).

Another abundant species was *Enterobacter cloacae* (5.58%) at the beginning of production (day 0). *E. cloacae* (8.63%) remained in the product for 14 days of maturation, and the presence of *Enterobacter aerogenes* (12.64%) was also observed at 14 days of maturation. The genus *Enterobacter* spp., found in both samples (days 0 and 14), is identified in various foods since it grows at a wide range of temperatures and ferments carbohydrates, producing organic acids. This characteristic leads to the development of off-flavors, gas production, and slime formation in meat products as metabolic by-products, causing damage to the final product (Feiner, 2006). The development of *E. cloacae* and *E. aerogenes* in the salami on day 14 is related to the decarboxylation of amino acids, potentially producing biogenic amines. High concentrations of biogenic amines, such as histidine formed by *E. aerogenes* and *E. cloacae*, when ingested, can lead to headaches and gastrointestinal issues for consumers (Durlu-Özkaya et al., 2001). Furthermore, in fermented sausages, the presence of these two species is typically low. When they are abundant, it is explained by possible inadequate raw material storage or incorrect fermentation, leading to increased decarboxylation during the early stages of production (Sarkadi, 2019). Therefore, it can be speculated that the artisanal process allowed for the development of these species up to day 14 (Figure 2).

Similarly to *E. cloacae* and *E. aerogenes*, the species *Citrobacter freudii*, which showed an increase from 3% to 10% in relative abundance over the initial 14 days of maturation in the salami samle, also exhibits characteristics of amino acid decarboxylation. *C. freundii* (18.42%) had the highest relative abundance among bacterial species present on day 14 (Figure 2). Bacteria belonging to *Citrobacter* spp. are also glucose fermenters, producing organic acids, and require a pH above 4.5 and water activity above 0.95 for their growth (Feiner, 2006). This explains the decrease in their population at the end of maturation on day 28. These microorganisms are good indicators of hygiene levels in food production, reflecting poor hygiene practices that may be associated with the entire salami production process (Feiner, 2006). Additionally, the species *Acinetobacter tandoii*, which represented 9.97% on day 14, has been reported to be important for cellulose fermentation in the intestines of termites (Van Dexter & Boopathy, 2019). Its presence in the salami may be due to contamination during production.

Aeromonas spp., abundantly present in the salami sample on day 14 (Figure 1), can also be directly linked to meat and fat handling conditions, carcass washing with contaminated water, as well as inadequate sanitation during product preparation (Stratev & Rusev, 2012). The development of *Aeromonas* spp. at the beginning of the fermentation process is related to pH and insufficient salt levels to inhibit their growth, but as the pH decreases, the growth of *Aeromonas* spp. tends to decrease. It's worth noting that several species in this genus are emerging agents of foodborne diseases and require attention when present in products ready for consumption, making it interesting to study the species present in the final product (Fontes et al., 2012). However, their presence on day 14 may not be problematic since their population was suppressed during fermentation, resulting in a relatively insignificant abundance by day 28 (Figure 1). *Aeromonas dhakensis* (6.9%), present on day 14, is considered an undesirable microorganism within this genus due to its potential pathogenicity (Chen et al., 2016) and may have originated from water used during processing or handling of the salamis during fermentation.

Throughout the fermentation process, LAB, formerly known as *Lactobacillus* (Zheng et al., 2020), developed. These bacteria were also present in large quantities on day 14 of the salami (Figure 1). They have the ability to ferment the sugars present, producing lactic acid, lowering the product's pH, and consequently dehydrating the meat fibers and accelerating this process. Their development contributes to controlling the growth of undesirable microorganisms, such as pathogens and spoilage microorganisms mentioned earlier, improves color, and imparts the characteristic acidic flavor to the final product (Senter, 2014).

At the end of the maturation process (day 28), despite the significant presence of *Companilactobacillus* spp. (*Companilactobacillus farciminis*, 9.77%), a higher abundance of *Staphylococcus* spp. (*Staphylococcus saprophyticus*, 62.57%) was observed (Figures 1 and 2) (Wang et al., 2018). Microorganisms like *Staphylococcus* spp., provided they are coagulase-negative, play a crucial role in the final stages of maturation, as they produce proteolytic and lipolytic enzymes that release low molecular weight compounds such as peptides, amino acids, aldehydes,

amines, and free fatty acids, resulting in changes to the aromatic profile of the final product (Cocolin & Rantsiou, 2012). *S. saprophyticus* is a common bacterium in animal-derived foods, and although it is coagulase-negative and does not reduce nitrite, it produces volatile compounds during fermentation, rapidly acidifying and contributing to the microbiological safety of the product (Sánchez Mainar et al., 2017). Its presence has been reported in traditional fermented sausages from Taiwan and artisanal salamis, often being one of the most abundant species (Charmpi et al., 2020; Tu et al., 2010). However, due to the pathogenic potential of the microorganism, its addition as a starter culture is not recommended, and its presence should be evaluated with caution.

On the 28th day of maturation, the species *C. farciminis* showed a significant relative abundance (9.77%). This species exhibits desirable characteristics with the potential for development throughout the maturation period, which determined its persistence from the 14th day (9.52%) to the last day. This bacterium belongs to the LAB group and is part of the natural microbiota of spontaneously fermented meat sausages (Połka et al., 2015; Tu et al., 2010). This bacterium plays an important role in this product as it is responsible for lowering the pH, which assists in the final microbiological safety of the sausages. This reaction occurs along with the production of bacteriocins, ensuring the stability and firmness of the sausage, in addition to producing volatile compounds and exhibiting proteolytic activity (Aspri & Tsaltas, 2020). Furthermore, they have the ability to reduce nitrate to nitrite, which aids in the initial formation of color, flavor, and odor and acts as a potential probiotic in the host. When administered in appropriate quantities, it can benefit the consumer and has the potential for application as a starter culture (Feldmann, 2015; Sayas-Barberá et al., 2012).

3.2 Fungal analysis

From the metataxonomic analysis, a total of 24,988 fungal reads were identified, with 9,665 of them at the beginning of salami maturation (day 0) and 7,923 and 7,400 reads at days 14 and 28 of maturation, respectively. Figure 1B presents the relative abundance of fungal genera throughout the maturation of artisanal salami.

On the production day (day 0), 11 genera were prominent, including *Yarrowia* spp. (24.96%), *Pichia* spp. (23.91%), *Fusarium* spp. (10.99%), *Candida* spp. (10.38%), and *Aspergillus* (4.15%), which were the most abundant in relative abundance (%). Among the fungal species detected, there was a significant fluctuation in their relative abundances (Table 2). On day 0, with a total of 9,665 reads, 17 prominent species were present. *Yarrowia lipolytica* (24.96%) was the most abundant, followed by *Pichia kudriavzevii* (19.54%), *Candida dubliniensis* (10.33%), and *Xerochrysium dermatitidis* (5.20%) (Figure 3).

Yeasts that are frequently found in fermented meat products are *Candida* spp. and *Y. lipolytica* (Gardini et al., 2001; Patrignani et al., 2007). Yeasts can cause an increase in pH and a decrease in lactic acid content in salami, contributing to the product's characteristics (Gardini et al., 2001). They can be added as flavor enhancers and stabilize the red color of fermented sausages (Olesen & Stahnke, 2000). Species of the *Pichia* spp. and *Candida* spp. genera produce various flavonoid compounds during maturation phases related to flavor and odor enhancement in fermented sausages (Wen et al., 2023). Patrignani et al. (2007) reported that salamis inoculated with *Y. lipolytica* strains showed faster and more significant reductions in water activity, and their presence on the sausage surface resulted in more pronounced proteolysis and lipolysis processes. Besides acting as a flavor source, yeasts can grow in high populations on the surface of dry-cured meat, making them eligible for a potential role as antagonists against undesirable fungi (Cano-García et al., 2013; Purriños et al., 2013).

Although filamentous fungi are more characteristic of the advanced maturation process, *X. dermatitidis* was also observed at the beginning of the process. *Xerochrysium* spp. species have been observed in fermented hams from southwestern Chinese regions and are closely related to the production of free amino acids (Lin et al., 2020). *X. dermatitidis* was initially discovered in dried meats, and its presence can lead to increased proteolysis, contributing to flavor development in the product (Li et al., 2022).

On day 14 of maturation, the most abundant genera were *Hyphopichia* spp. (73.85%) and *Yarrowia* spp. (18.81%), with *Candida* spp. (2.75%) also being notable due to its significance in the literature (Figure 1B). After 14 days of maturation, there was a noticeable decrease in the number of reads of fungal species compared to the previous measurement. At this point, the total reads of species were 7,923 (an 18% drop compared to day 0), and the relative abundance by species also changed, reducing to only five species, with *Hyphopichia burtonii* (73.85%) and *Y. lipolytica* (18.81%) being the most abundant (Figure 3). The yeast *H. burtonii* has been reported to be important for aroma development and essential nutrient production in fermented products such as vinegar and fermented peppers (Xu et al., 2021). The initial presence of this species in the salami may be associated with the addition of vinegar to the product preparation. For the development of *H. burtonii*, temperature is more critical than pH, with an optimal temperature of approximately 30°C (Burgain et al., 2015; Debonne et al., 2021). The fact that the salamis matured in this temperature range explains the significant development of the species. Additionally, Burgain et al. (2015) reported better growth of *H. burtonii* at low water activity, which aligns with the species' progression throughout the product's maturation phase, where salami dehydration occurs. *H. burtonii* is a food spoilage organism as it has been shown to produce styrene, resulting in an unpleasant taste in fermented bakery products, but it is also a source of cheese notes in cured foods (Groenewald & Smith, 2010). Therefore, it is necessary to determine and confirm which strains produce flavors to ensure greater safety in the production of fermented foods in which the species is prominent.

On the final day of maturation (day 28), the relative abundances were *Hyphopichia* spp. (73.73%), *Aspergillus* spp. (14.61%), *Wallemia* spp. (6.30%), and *Yarrowia* spp. (4.47%) (Figure 1B). At the end of the maturation period (day 28), there was again a decrease in the total number of reads to 7,400 (a 23.44% decrease compared to day 0). The prominent species

Table 2. Number of reads of fungi species identified throughout the maturation period of artisanal salami.

Species	Total	Day 0	Day 14	Day 28
Acremonium antarcticum	13	12	1	0
Aspergillus amstelodami	386	329	10	47
Aspergillus candidus	5	0	0	5
Aspergillus cibarius	866	21	142	703
Aspergillus europaeus	15	0	0	15
Aspergillus melleus	5	5	0	0
Aspergillus nidulans	22	21	1	0
Aspergillus restrictus	26	9	3	14
Aspergillus ruber	321	16	14	291
Aspergillus versicolor	6	0	0	6
Auricularia cornea	6	6	0	0
Candida dubliniensis	1,017	998	17	2
Candida metapsilosis	129	2	116	11
Candida orthopsilosis	99	3	85	11
Cladosporium cladosporioides	80	79	1	0
Cladosporium sphaerospermum	15	5	1	9
Clavispora lusitaniae	18	18	0	0
Colletotrichum capsici	26	25	1	0
Colletotrichum karsti	21	21	0	0
Colletotrichum siamense	58	56	2	0
Cryptococcus aff. taibaiensis IMUFRJ 51982	31	31	0	0
Cryptococcus sp. LRB-2012a	9	9	0	0
Cryptococcus sp. SJ4L02	18	18	0	0
Cutaneotrichosporon jirovecii	9	9	0	0
Cyberlindnera fabianii	47	47	0	0
Cyberlindnera rhodanensis	10	10	0	0
Cyberlindnera veronae	11	11	0	0
Diutina mesorugosa	16	16	0	0
Diutina rugosa	11	11	0	0
Furcasterigmium furcatum	35	35	0	0
Fusarium delphinoides	32	31	1	0
Fusarium dimerum	40	40	0	0
Fusarium equiseti	151	151	0	0
Fusarium fujikuroi	147	145	2	0
Fusarium lateritium	5	5	0	0
Fusarium nectrioides	34	32	2	0
Fusarium oxysporum	5	5	0	0
Fusarium solani	151	150	1	0
Galactomyces reessii	261	258	2	1
Geotrichum candidum	12	12	0	0
Hannaella luteola	8	8	0	0
Hannaella sinensis	8	8	0	0
Hyphopichia burtonii	11,530	223	5,851	5,456
Kluyveromyces marxianus	6	6	0	0
Kodamaea ohmeri	32	32	0	0
Lasiodiplodia pseudotheobromae	7	7	0	0
Meyerozyma carpophila	224	173	45	6
Nigrospora oryzae	8	8	0	0
Penicillium citrinum	6	5	0	1
Pichia kluyveri	417	407	7	3
Pichia kudriavzevii	1,930	1,889	41	0
Pichia manshurica	15	15	0	0
Pyrenochaetopsis microspora	7	7	0	0
Rhizopus oryzae	9	9	0	0
Rhodosporidiobolus fluvialis	25	25	0	0
Rhodosporidiobolus ruineniae	7	7	0	0
Rhodotorula paludigena	23	22	1	0
Saccharomyces cerevisiae	9	8	1	0
Sphaeronaemella fimicola	31	31	0	0
Sporobolomyces sp. Vega180	7	7	0	0
Trichosporon insectorum	139	138	1	0
Wallemia mellicola	831	324	42	465
Wallemia sebi	16	15	0	1
Wallemia sp. SJ-2014	13	13	0	0
Wallrothiella subiculosa	5	5	0	0
Wickerhamiella azyma	9	9	0	0
Wickerhamomyces anomalus	55	53	2	0
Wickerhamomyces sp. LCF-15	26	26	0	0
Xerochrysium dermatitidis	517	503	9	5
Xeromyces bisporus	7	7	0	0
Yarrowia lipolytica	4,233	2,412	1,490	331
[Candida] intermedia	45	41	4	0
[Candida] palmioleophila	70	51	10	9
[Candida] quercitrusa	31	30	1	0
[Candida] stellimalicola	35	35	0	0
Leaf litter ascomycete strain its354	10	10	0	0
Uncultured *Galactomyces*	468	444	16	8
Total for the sample	24,988	9,665	7,923	7,400

on day 28 continued with the predominance of *H. burtonii* (73.73%), along with the participation of *Aspergillus cibarius* (9.50%), *Wallemia mellicola* (6.28%), *Y. lipolytica* (4.47%), and *Aspergillus ruber* (3.93%) (Figure 3).

Throughout the fermentation process, the surface of the fermented sausage becomes colonized by filamentous fungi, which have the ability to thrive in various environments and substrates (Magistà et al., 2017). In this type of fermented product, filamentous fungi play an important role in the production process as they can contribute to the development of specific flavors and aromas due to their lipolytic and proteolytic activities (Sonjak et al., 2011). In the microbiota of dry-cured meat products, fungi of the genus *Aspergillus* spp. are responsible for controlling light incidence on the sausage and the passage of oxygen (Cence, 2016; Schmitt, 2017), exerting an antioxidant effect, protecting against rancidity, and maintaining color. They give the sausage its typical appearance, allowing the development of a positive microclimate on the surface to prevent sticky or viscous characteristics (Visagie et al., 2014). On the 28th day of maturation (Figure 3), the species *A. cibarius* and *A. ruber* were highlighted. The former is a fungus characteristic of meju fermentation, the initial fermented material used for traditional Korean soy sauce and soybean paste (Hong et al., 2015). Meanwhile, *A. ruber* is often related to the fermentation of Jamun leaves,

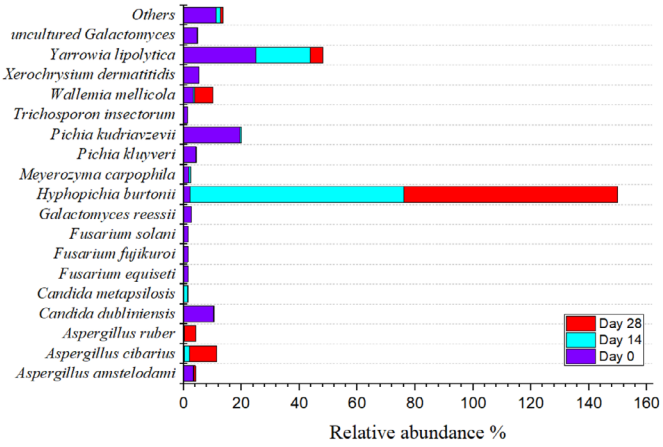

Figure 3. Relative abundance (%) of fungi species on days 0, 14, and 28 of artisanal salami maturation.

producing tannases, which are cheap and valued substrates in India (Kumar et al., 2007).

Wallemia spp. is also present at the end of the studied maturation period in this study. Species of the *Wallemia* genus have shown adaptive survival capabilities in low water activity (Tian et al., 2022), explaining their prominence at the end of maturation. Furthermore, *W. mellicola* can contaminate foods preserved with high levels of salt or sugar (Jančič et al., 2016). I creasing the NaCl concentration from 5% to 15% in the growth medium of *Wallemia* spp. increased the production of toxic metabolites by the species (Jančič et al., 2016). This capacity to produce toxins may have served for the microbiological control of other species during maturation.

3.3 Ecological interactions between fungi and bacteria during maturation

Based on the results and discussions presented regarding the microbiological ecosystem, it is possible to make some observations and suggest possible microecological interactions that occurred during the maturation period. Among the possible control measures that were established, the following stand out:

- changes in pH over the course of the period;
- dehydration of the sausages over the days;
- a decrease in the supply of glucose, proteins, and complex lipids;
- production and removal of metabolites and toxic residues.

In the middle of maturation, the presence of certain microorganisms may have acted as a means of controlling microbial colonies, given that pH (I) is a determining factor for the optimal activity of proteins (Kress-Rogers, 1991). LAB species ferment sugars into lactic acid, contributing to the maintenance of a pH in the range of 5.0–4.8 (Franciosa et al., 2018). This acidification process plays a fundamental role in preventing pathogen growth and product degradation (Franciosa et al., 2018). Similarly, fungi and yeast can produce metabolites that alter the pH of the environment, acting as a method of selection for microbial growth. The increase in pH and decrease in lactic acid content in the sausage could have been caused by yeast, favoring the development of *Y. lipolytica,* which has optimal lipolytic activity at pH 5.5 (Gardini et al., 2001).

Another critical factor for microbial growth is water activity (II) (Pandey, 1992). Water is probably the most important factor governing microbial spoilage in food, and the concept of water activity is valuable as the measured values typically correlate with the potential for growth and metabolic activity (Chirife et al., 1996). Given this, the dehydration of the sausage during maturation is a crucial factor in controlling microbiological biodiversity, as evidenced by a 23% reduction in fungal reads from before maturation to day 28. For example, fungi such as those belonging to the genera *Aspergillus* and *Penicillium* are sensitive to changes in water activity (Mannaa & Kim, 2017), a characteristic that justifies the decline in the abundance of their populations during maturation (Figure 3).

The degradation of macromolecules such as complex proteins and long-chain lipids into amino acids and simple fatty acids can act as a determining factor for the development of species that use these molecules in their metabolism (III). The development of many species may have acted in the sausage as a factor competing for nutrients, contributing to a shortage of certain substances and the death of species dependent on them. Examples of microorganisms that may have acted as consumers of complex nutrients are coagulase-negative cocci species, involved in proteolytic and lipolytic processes that are crucial for the development of the final organoleptic characteristics (Hammes & Hertel, 1998). This metabolism was observed in this study for *S. saprophyticus. Yeasts* like *Y. lipolytica, H. burtonii,* and *X. dermatitidis,* as well as filamentous fungi like *Penicillium,* have proteolytic and lipolytic activities crucial for the fermentation of meat products (Groenewald & Smith, 2010; Li et al., 2022; Patrignani et al., 2007).

In fermented meat products, the accumulation of specific metabolites, such as lactic acid, acetic acid, formic acid, ethanol, ammonia, fatty acids, hydrogen peroxide, acetaldehyde, and bacteriocins, can act as a source of biological control, inhibiting the growth of certain microorganisms (IV) (Hugas & Monfort, 1997). Strains of all LAB genera were identified as bacteriocin producers, and these bacteria are important in the meat microbiota composition, acting against bacteria closely related to them (Lücke, 2000). Bacteriocins enhance the competitiveness of a strain for nutrients during fermentation (Hugas & Monfort, 1997), which suggests that they are a determining factor in the microbiome composition. The presence of various bacteriocin-producing bacteria may have modulated the sausage ecosystem, contributing to the final abundance of species.

Yeasts can produce metabolites that have a significant suppressive effect on the expression of genes related to mycotoxin biosynthesis and/or inhibit the growth of filamentous fungi (Pfliegler et al., 2015). Strains of *Pichia kluyveri* produced volatile organic compounds that inhibited the growth of *Aspergillus* species and blocked the production of one of the most important mycotoxins, ochratoxin A (OTA), during coffee production

(Masoud et al., 2005). In a study on the biocontrol activity of native yeast flora in dry-cured ham, the species *Debaryomyces, Candida,* and *Hyphopichia* inhibited OTA biosynthesis, and native yeasts also had an antagonistic effect on the growth of *Penicillium nordicum*. Additionally, *H. burtonii* and *Candida zeylanoides* were the most effective in both reducing the growth and OTA biosynthesis of the fungus (Kabak & Dobson, 2009). Therefore, the fungi and yeasts in the sausage may have produced mycotoxins and metabolites that influenced the entire microbiome during maturation. These interactions warrant further investigation in future studies.

4 CONCLUSIONS

Fermented sausages are extremely complex microbiological ecosystems, harboring a wide range of bacterial, fungal, and yeast genera and species on their surface and within the sausage. This microbiome develops various types of interactions that modulate the metabolisms of all these microorganisms in a highly intricate manner. Thus, further research on salami analyses is needed to gain a deeper understanding of the microbiota of fermented meat products and determine the complex ecological relationships discussed in our work.

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