Current Research in Microbial Sciences 9 (2025) 100516

Contents lists available at ScienceDirect

Current Research in Microbial Sciences

journal homepage: www.sciencedirect.com/journal/current-research-in-microbial-sciences

Exploring diversity and functional contribution of the microbiome of traditional Italian dry-cured hams

Giulia Longhi [a], Chiara Tarracchini [a], Leonora Angelini [b], Rosaria Anzalone [b], Alice Viappiani [b], Marco Ventura [a,c], Christian Milani [a,c,*], Francesca Turroni [a,c,*]

[a] Laboratory of Probiogenomics, Department of Chemistry, Life Sciences, and Environmental Sustainability, University of Parma, Parma, Italy
[b] GenProbio srl, 43124 Parma, Italy
[c] Microbiome Research Hub, University of Parma, Parma, Italy

ARTICLE INFO

Keywords:
Dry-cured ham
Food microbiota
Microbiome
Genomics
Metagenomics

ABSTRACT

Traditional dry-cured hams host diverse microbial communities; however, their taxonomic composition, functional capacity, and potential interactions with the human gut remain poorly understood. This study aimed to provide a comprehensive characterization of the microbiota associated with Italian Protected Designation of Origin (PDO) dry-cured hams and to investigate their functional relevance in the food matrix and under simulated intestinal conditions. A total of 96 samples, representing different geographical origins and maturation stages, were analyzed using metagenomics approaches. A conserved microbial core dominated by *Staphylococcus equorum* (prevalence 80 %) was identified, accompanied by accessory taxa such as *Tetragenococcus halophilus* (62 %) and *Leuconostoc carnosum* (10 %). Cluster analyses revealed substantial variability across samples, with community structures influenced more by producer-specific factors than by product type or ripening stage. Functional metagenomics investigation highlighted the presence of metabolic pathways associated with amino acid degradation, carbohydrate metabolism, and lipid transformation, supporting a role for ham-associated microbes in flavor and texture development. Furthermore, cultivation in a simulated gut environment showed a marked reshaping of the microbial community, with low-abundance taxa, including *Bacillus* spp. and *Lactococcus lactis*, proliferating under intestinal-like conditions, while the dominance of *S. equorum* was reduced. Our findings showed that the microbiota of dry-cured ham not only drives key sensory qualities of the product but also comprises a reservoir of live microorganisms capable of tolerating the gut-like conditions. These results highlight the dual role of foodborne microbiota in shaping both food properties and potential interactions with the human host, underscoring the need for further *in vivo* investigations.

Introduction

Over the last few decades, microbiome research has revealed the intricate and dynamic interactions between the human host and the microbial communities inhabiting the gastrointestinal tract. Among the many factors influencing gut microbiota composition, diet plays a pivotal role, providing nutrients and prebiotics and serving as a source of live and metabolically active microorganisms (Zhang et al., 2025a; Bibbo et al., 2016). These ingested microbes may influence the gut ecosystem both transiently and, in some cases, more persistently, with potential impacts on host immunity, metabolism, and microbial diversity (Roselli et al., 2021). This growing evidence has led to the emerging concept of foodborne microorganisms as active modulators of the human gut microbiome dynamics (Lugli et al., 2022; Milani et al., 2019).

Traditional fermented foods, such as yogurt, kefir, kimchi, tempeh, aged cheeses, and dry-cured meats, are particularly rich in viable microorganisms, including species of *Lactobacillus, Leuconostoc, Staphylococcus, Brevibacterium*, and *Tetragenococcus* (Wastyk et al., 2021). Functional metagenomic studies have suggested that these microbes may indirectly modulate the gut environment through the production of organic acids, bacteriocins, and hydrolytic enzymes (Umu et al., 2017; Fontana et al., 2024; Milani et al., 2025).

In this context, dry-cured hams, especially traditional Italian

* Corresponding author at: Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma, Parco Area delle Scienze 11a, 43124 Parma, Italy.
E-mail addresses: christian.milani@unipr.it (C. Milani), francesca.turroni@unipr.it (F. Turroni).

https://doi.org/10.1016/j.crmicr.2025.100516

Available online 25 November 2025

varieties such as Parma-, San Daniele- and Tuscan-hams, represent emblematic examples of long-ripened meat products shaped by complex biochemical and microbial components. Italian dry-cured hams are produced from selected pigs following a strictly regulated process that includes salting, resting, drying, and prolonged ripening under controlled temperature and humidity without any heat treatment. Over 12–24 months, gradual dehydration, salt diffusion, proteolysis and lipolysis generate a unique physicochemical environment that shapes both sensory quality and microbial ecology (Martinez-Onandi et al., 2019; Toscano et al., 2024; Piasentier et al., 2021). The resulting low water activity (a_w) and high NaCl concentration create a selective niche dominated by halotolerant and proteolytic taxa, mainly *Staphylococcus, Tetragenococcus, Brevibacterium*, and *Corynebacterium* (Toscano et al., 2024; Cerrato et al., 2022). These indigenous microorganisms play an important role in flavor development, as their proteolytic and lipolytic activities release peptides, free amino acids, and volatile compounds responsible for the characteristic aroma and taste of dry-cured hams (Li et al., 2025a; Zhang et al., 2025b; Wang et al., 2025). Despite increasing interest in food microbiomes, the ecological and functional roles of dry-cured ham microorganisms remain poorly understood, particularly regarding their potential interaction with the human gut.

Recent applications of high-throughput metagenomics attempts have revealed a complex ham-associated microbiota shaped by region, processing, and ripening conditions (Toscano et al., 2024; Mu et al., 2019). However, whether these microorganisms can survive or tolerate gut-like environments, even transiently, has not been explored so far in great detail. Addressing this gap could provide novel insights into the ecological relevance of foodborne microbes and their potential, albeit temporary, contribution to microbial dynamics. This study aimed to comprehensively characterize the microbial functional potential of traditional Italian dry-cured hams, thereby addressing existing gaps in the understanding of their functional and metabolic profiles. We analyzed the taxonomic composition of different ham types and identified a conserved core community alongside product-specific differences. In addition, we explored how microbial composition varied according to maturation stage and geographical origin through statistical analyses of associated metadata, and we evaluated the intestinal adaptability of ham-associated microorganisms using batch culture experiments in a simulated gut environment. Shotgun metagenomic analyses further enable the identification of key metabolic pathways related to flavor and texture formation. Altogether, these approaches provide an integrated view of the ecological, functional, and adaptive traits of the dry-cured ham microbiota.

Materials and methods

Ham sample collection

A total of 96 Italian dry-cured ham samples were collected, mostly consisting of Parma ham (approximately 80 %), obtained through a collaboration with the Parma Ham Consortium, and sourced from different geographical zones within the Parma province and representing a range of local producers. Additional samples (the remaining 20 %) included San Daniele, Carpegna, Norcia, and Tuscan hams, covering the diversity of traditional PDO dry-cured hams from central and northern Italy. The samples encompassed various maturation times, reflecting different ripening stages. More details regarding the variety of ham samples are reported in Table S1. All collected hams were produced without thermal treatment, preserving the viability of naturally occurring microorganisms. No precise information was recorded regarding temperature or humidity during ripening, as these parameters may vary according to producer-specific practices. However, for Parma dry-cured ham, technological parameters are strongly standardized under the PDO denomination, which prescribes controlled conditions of temperature, humidity, and duration for each processing phase. Salting is performed at 1–4 °C and 80–90 % relative humidity, followed by a resting period at 1–5 °C and 70–80 % Relative Humidity (RH), a pre-ripening phase at 15–23 °C and 60–80 % RH, and a final ripening stage lasting at least 12 months under 15–20 °C and 60–80 % RH, with regulated airflow and surface treatment with lard paste. These standardized conditions minimize variability, although slight adjustments among producers (*e.g.*, microclimate, airflow management) may contribute to subtle differences in microbial and biochemical profiles. In addition to these technological parameters, intrinsic characteristics of the raw material further influence the final product, introducing variability that is specific to each individual ham. Approximately 200 g of each ham was collected, vacuum-packed, and kept on ice during transport to the laboratory. Afterward, the samples were stored at −80 °C until further processing.

Bacterial DNA extraction and microbial ITS profiling

To avoid contamination from the outer surface, a fixed amount of 1 g of ham, corresponding to the central portion of each sample, was homogenized with 9 mL of Phosphate Buffered Saline (PBS; pH 6.5) to minimize the impact of fat and connective tissue. All sample handling and homogenization procedures were performed under sterile conditions using aseptic tools and a laminar flow cabinet to prevent external microbial contamination. Subsequently, 1.5 mL of each resuspended ham sample was subjected to bacterial DNA extraction using a DNeasy PowerFood Microbial Kit according to the manufacturer's instructions (Qiagen, Germany). Then, DNA concentration and purity were assessed using a fluorometric Qubit quantification system (Life Technologies, USA). ITS sequences were amplified from extracted DNA using the primer pair UNI_ITS_fw (5′-KRGGRYKAAGTCGTAACAAG-3′) and UNI_ITS_rv (5′-TTTTCRYCTTTCCCTCACGG-3′), targeting the entire spacer region between the 16S rRNA and 23S rRNA genes within the rRNA locus. The amplification was carried out using GoTaq G2 Hot Start polymerase (Promega, USA) on a Verity thermocycler (Applied Biosystems, USA) according to the following protocol: 95 °C for 10 min, followed by 32 cycles of 95 °C for 1 min, 52 °C for 1 min, and 72 °C for 1 min and a final step of 72 °C for 5 min. The integrity of PCR amplicons was analyzed by gel electrophoresis. DNA products obtained following PCR-mediated amplification of the ITS rRNA gene sequences were purified using a magnetic purification step with Agencourt AMPure XP DNA purification beads (Beckman Coulter Genomics GmbH, Bernried, Germany) to remove primer dimers. DNA concentration of the amplified sequence library was determined by a fluorometric Qubit quantification system (Life Technologies, USA). Amplicons were diluted to a concentration of 4 nM, and 5 µl quantities of each diluted DNA amplicon sample were mixed to prepare the pooled final library. Sequencing was performed using NextSeq 2000 and MiSeq platforms (Illumina, San Diego, *CA*).

Metagenomic data processing

Taxonomic profiling of sequenced reads was performed with the METAnnotatorX2 bioinformatics platform (Milani et al., 2021; Milani et al., 2018). In detail, the raw data in fastq format were submitted to quality filtering with removal of reads with an average quality <25. Subsequently, host DNA was removed by reads mapping to the *Sus scrofa* genome. Finally, the retained sequences were used as input to perform a MegaBLAST local reads alignment to a preprocessed database of prokaryotic sequences. Reads showing a nucleotide identity >94 % to the genomes included in the database were classified at the species level, while if a lower percentage identity was detected, they were classified at the genus level as undefined species. Functional profiling of sequenced reads was performed with the METAnnotatorX2 bioinformatics platform (Milani et al., 2021; Milani et al., 2018) against the Rhea-Annotated Reactions Database (Bansal et al., 2022), with thresholds set at −query-cover 80, −evalue 1×10^{-8}, and −max-target-seqs 1. This approach enabled the identification of enzymatic functions related to

amino acid degradation, carbohydrate metabolism, and lipid transformations; processes potentially linked to flavor and texture development in dry-cured hams. Protein domain validation was performed with InterProScan 5.68–100.0 (Quevillon et al., 2005) to ensure stringent filtering and reduce redundancy by retaining only conserved, functionally relevant domains. Functional abundance was normalized to the number of mapped reads, and only functions exceeding 0.1 % relative abundance were considered for downstream analysis.

Shotgun metagenomic sequencing and functional analysis

Two representative ham samples (18-month-ripened, cluster 7) were subjected to deep shotgun sequencing to ensure higher coverage and enable a more detailed functional characterization of the ham-associated microbiota. DNA was prepared using the Illumina Nextera XT DNA Library Preparation Kit. Briefly, samples were enzymatically fragmented to 550–650 bp with a BioRuptor machine (Diagenode, Belgium), barcoded, and purified using Agencourt AMPure XP DNA purification beads (Beckman Coulter Genomics GmbH, Bernried, Germany). DNA concentration was quantified using the Qubit fluorometric system (Life Technologies, USA) and analyzed on a 2200 TapeStation instrument (Agilent Technologies, USA), and normalized to 4 nM. Sequencing was performed on an Illumina NextSeq 2000 platform (Illumina Inc., San Diego, USA). Paired-end reads were quality filtered using fastq-mcf (mean quality score >20 and read length >100 bp). Functional and taxonomic annotation was carried out with METAnnotatorX2 (Li et al., 2022), as described above.

Cultivation of ham microbiota in a simulated intestinal environment

To investigate the ability of ham-associated microbes to grow in conditions resembling the human intestinal tract, portions of 18-month-ripened dry-cured ham were incubated in a gut environment-simulating medium (GESM) previously described in the literature (Macfarlane et al., 1998; Milani et al., 2025). Specifically, the culture medium consisted of the following constituents (g liter−1) in distilled water: starch 5.0; pectin (citrus) 2.0; guar gum 1.0; mucin (porcine gastric type III) 4.0; xylan (oat spelt) 2.0; arabinogalactan (larch wood) 2.0; inulin 1.0; casein acid hydrolysate 3.0; peptone water 5.0; Bacto™ tryptone 5.0; bile salts 0.4; yeast extract 4.5; $FeSO_4 \times 7H2O$ 0.005; $MnCL_2 \times 4H_2O$ 0.2; NaCl 4.5; KCl 4.5; KH_2PO_4 0.5; $MgSO_4$ 0.61; $CaCl2 \times 2H_2O$ 0.15; $NaHCO_3$ 1.5; L-Cysteine HCl 0.8; hemin 0.05; Tween 80 1.0; vitamin solution 1.0. This medium was formulated to mimic key features of the intestinal niche, including the presence of bile salts and mucin, and was maintained under tightly controlled conditions of temperature, pH, and anaerobiosis (Macfarlane et al., 1998; Fontana et al., 2023). It should be noted that the GESM experiment was performed in a controlled *in vitro* system without intestinal cells or immune components; therefore, it cannot demonstrate microbial colonization or persistence in the human gut, but only the potential survival and metabolic activity of ham-associated microorganisms under simulated gastrointestinal conditions. For each assay, 0.1 % (wt/vol) of finely homogenized ham was inoculated into 30 mL of GESM, and incubations were carried out in triplicate at 37 °C under anaerobic conditions. Samples were handled under sterile conditions throughout to minimize external contamination. After 16 h of growth, batch cultivations were processed for bacterial DNA extraction and subjected to shallow shotgun sequencing.

Statistical analysis

ORIGIN 2023 (https://www.originlab.com/2023) and R-Studios were used to compute statistical analyses. For taxonomical, and functional input data was preprocessed and transformed in a Bray Curtis dissimilarity matrix with vegdist function (from vegan_2.5–7). PERMANOVA and ANOSIM analyses were performed on R-studios using 999 permutations to assess p-values for cluster differences in PCoA with adonis2 package (from vegan_2.5–7). The cumulative contribution rate below 50 % reflects the high intrinsic variability of the microbial dataset. The main clustering patterns observed were statistically supported and consistent with metadata-driven analyses, confirming the robustness of the results. Clusters representing <2 % of the total samples were excluded from the PCoA analysis.

When FDR correction was applied, the Benjamini-Hochberg or Bonferroni approach was used on R-studios through p.adjust function (from base package stats).

Availability of data and materials

Samples of the 96 ham samples and batch growth cultivations can be retrieved from NCBI under bioproject PRJNA1338105.

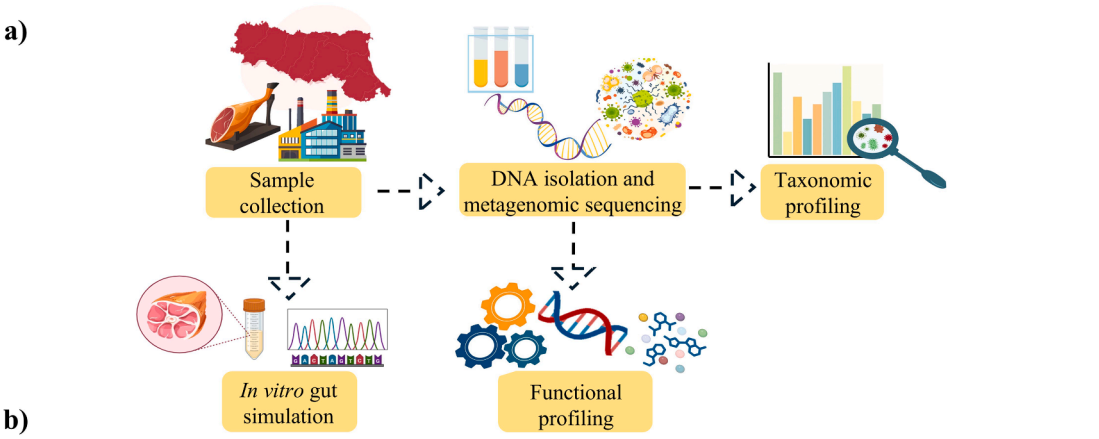
Results and discussion

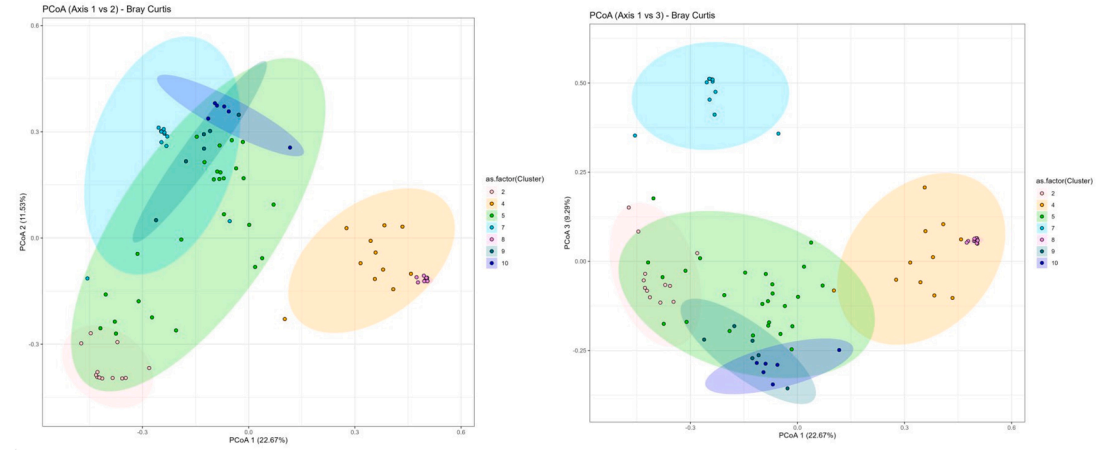
Decoding the microbial composition of traditional Italian dry-cured hams

To characterize the microbiota associated with traditional Italian dry-cured hams, we collected a total of 96 samples representative of the main PDO (Protected Designation of Origin) hams produced across central and northern Italy (Fig. 1a). The dataset mainly consisted of Parma ham samples, with a smaller subset including San Daniele, Carpegna, Norcia, and Tuscan hams, all produced according to strict geographic and procedural guidelines. Samples were obtained from a wide range of production contexts, encompassing both local producers who follow traditional artisanal methods and those employing more industrialized processing approaches (Table S1). However, traditionally produced artisanal hams are increasingly rare, resulting in a higher proportion of samples from industrial producers. All hams were produced using traditional dry-curing methods without thermal treatment, in accordance with the respective production specifications. The dataset covered a broad range of maturation times, from 14 to 34 months (Table S1). Microbial DNA extracted from the collected samples was subjected to high-throughput sequencing targeting the ITS region of the bacterial rRNA operon, using optimized universal primers previously described for their ability to achieve (sub)species-level taxonomic resolution across various matrices, including food samples (Fig. 1a) (Milani et al., 2020). This approach enabled the reconstruction of detailed taxonomic profiles, extending beyond the genus level, across the whole sample set. The resulting microbial profiles offered a comprehensive overview of the bacterial core (prevalence ranging from 80 % to 100 %) and accessory members (prevalence between 45 % and 80 %), as observed across different ham types and curing conditions (Table S2). Notably, *Staphylococcus equorum* represents the entire core microbiota, being the only species detected with a prevalence greater than 80 % (Table S2). The high abundance of this species likely reflects its ability to tolerate saline stress and metabolically adapt to the low-a_w environment of dry-cured hams, together with its frequent presence in traditional processing settings (Landeta et al., 2013; Leroy et al., 2009; Stavropoulou et al., 2018). Among the accessory microbial members of the ham microbiota, the most represented were different species belonging to the *Tetragenococcus* genus, such as *Tetragenococcus halophilus* (prevalence around 62 %) and *Tetragenococcus koreensis* (45.1 %) (Table S2), which are frequently associated with the maturation and flavor development of fermented and salted food products (Tamang et al., 2016).

Moreover, less common occurring bacterial taxa, each with a prevalence of up to 10 %, included *Leuconostoc carnosum*, as well as various species of *Streptococcus, Bacillus, Lactobacillus* (*e.g.*, L. *iners*, L. *crispatus*), *Bacteroides*, and *Carnobacterium* genera (Table S2).

Comparative analyses with other traditional dry-cured hams from different regions reveal both shared and distinctive microbial features. Studies on Spanish and Iberian hams have consistently reported the predominance of *Staphylococcus* spp., particularly *S. equorum* and *S. xylosus*, which thrive under high NaCl concentration and reduced a_w,





c)

Bacterial species	Cluster 2	Cluster 4	Cluster 5	Cluster 7	Cluster 8	Cluster 9	Cluster 10
Tetragenococcus	7.08%		2.99%	66.55%			
Tetragenococcus koreensis				10.17%			
Tetragenococcus halophilus	83.00%	3.00%	13.97%	5.08%		3.05%	
Staphylococcus equorum	3.34%	53.69%	5.07%	2.37%	93.76%		4.72%
Bacillus amyloliquefaciens						45.97%	
Bacillus inaquosorum			2.18%			8.49%	6.38%
Cutibacterium acnes						6.17%	
Bacillus spp.						5.72%	
Lactobacillus crispatus		3.01%	4.28%			5.47%	13.13%
Bacillus velezensis						4.79%	
Lactobacillus iners						3.02%	53.14%
Cutibacterium spp.							3.66%
Lactobacillus spp.			6.45%				3.25%
Streptococcus spp.			2.65%				2.80%
Lactobacillus amylovorus							2.66%
Segatella hominis							
Pseudomonas spp.			3.32%				
Pseudomonas canadensis							
Staphylococcus xylosus			2.18%				
Fructobacillus spp.			3.16%				
Enterococcus spp.			2.60%				
Ligilactobacillus acidipiscis			2.34%				
Pseudomonas fragi			2.28%				
Kocuria palustris			2.24%				
Species below 2%	6.58%	33.29%	44.30%	15.84%	6.24%	17.30%	10.26%

Relative abundance

0% 2% 100%

Fig. 1. Microbial composition and diversity in traditional Italian dry-cured hams. Panel (a) illustrates a schematic representation of the study's workflow. Panel (b) shows the principal coordinate analysis (PCoA) based on the Bray-Curtis dissimilarity matrix of species-level microbial profiles, showing seven distinct clusters of ham samples based on beta-diversity. Clusters did not clearly correspond to PDO denomination or maturation stage, suggesting a predominant influence of producer-dependent factors and processing environments. Panel (c) depicts a heat map illustrating the relative abundance of bacterial species with an average abundance above 2 %. Species are listed on the left, and sample clusters are shown across the top. Each cluster corresponds to a group of ham samples sharing a similar taxonomic profile. The color gradient represents the relative abundance of each species within the corresponding cluster, allowing visualization of taxonomic differences among clusters.

similar to our findings in Italian PDO hams (Martinez-Onandi et al., 2017; Martinez-Onandi et al., 2019). In contrast, recent studies on Chinese dry-cured hams have identified a more diverse bacterial community, including *Halomonas, Pseudomonas, Psychrobacter, Alistipes*, and *Bacteroides*, reflecting differences in raw materials, salt concentration, and environmental curing conditions (Qin et al., 2024; Luo and Shen, 2025). Overall, the composition of ham-associated microbial communities appears to be shared by a combination of geographic, technological, and environmental factors. While halotolerant genera such as *Staphylococcus* and *Tetragenococcus* are consistently found in European hams due to similar curing practices, the broader microbial diversity observed in Asian products may result from distinct processing techniques and local microbial ecosystems. This variability underscores the complexity and adaptability of microbial communities in traditional dry-cured meat products; however, the reduced availability of artisanal hams limits the possibility of this study to fully capture the extent of the Italian ham microbiota complexity.

Microbial diversity and cluster analysis in dry-cured hams

Microbial variability at the species level was investigated across ham samples. Interestingly, beta-diversity analysis based on species-level taxonomic profiles, visualized through a two-dimensional principal coordinate analysis (PCoA), revealed seven distinct clusters of dry-cured ham samples according to their microbial composition (Fig. 1b, 1c). However, these clusters did not align clearly with specific product types or maturation stages, suggesting that the observed variability likely reflects the intrinsic heterogeneity of dry-cured hams, influenced by producer practices, raw material characteristics, and local curing microenvironmental conditions (Table S1). Interestingly, a similar pattern has been reported in traditional cheeses, where microbiota-based clustering often fails to correspond strictly with product denomination or ripening stage, highlighting instead the predominant influence of producer-dependent factors and local processing environments (Fontana et al., 2023; Reuben et al., 2023; De Filippis et al., 2014; Milani et al., 2019). In terms of alpha-diversity, species richness varied substantially across clusters, with some groups hosting complex microbial communities while others were dominated by only a few taxa (Fig. 1c) (Table S3). Among these, cluster 5 emerged as the most abundant group, comprising 26.5 % of samples derived from various types of PDO dry-cured hams, mostly produced using artisanal methods within the same geographical area (Table S1, S3). Notably, this cluster not only represented the largest proportion of samples but also exhibited the greatest species richness (287 species), underscoring its particularly diverse microbial community, with only 14 bacterial species exceeding a 2 % relative abundance. Specifically, *T. halophilus* dominated, reaching an average relative abundance of over 13.9 %, while the remaining species ranged from 2.1 % (*Bacillus inaquosorum, Staphylococcus xylosus*) to 6.4 % (*Lactobacillus* spp.), comprising various species (Table S3). This microbial configuration likely reflects the selective pressures of artisanal processing environments, which favor the growth of salt-tolerant and fermentative bacteria. Many of the observed taxa, such as *Lactobacillus, Tetragenococcus, Enterococcus*, and *Staphylococcus* spp., are known for their proteolytic and fermentative roles, contributing to flavor development during maturation (Toledano et al., 2011). These microorganisms secrete proteases and lipases hydrolyzing muscle proteins and lipids into smaller compounds, including peptides, free amino acids, and free fatty acids, which subsequently undergo oxidative and esterification reactions leading to the formation of volatile compounds responsible for the characteristic aroma and taste of dry-cured hams (Toldra and Flores, 1998; Toldra et al., 2020; Harkouss et al., 2012). *S. equorum* and *T. halophilus*, in particular, have been reported to play a key role in flavor generation through their catabolic activity on branched-chain amino acids and lipid-derived substrates (Pugliese et al., 2010; Gutsche et al., 2012; Smit et al., 2004). Others, including *Pseudomonas, Bacillus*, and *Kocuria*, exhibit metabolic versatility and tolerance to oxidative stress, supporting their persistence in oxygen-variable niches (Shi et al., 2021; Kolbeck et al., 2021; Liu et al., 2024). Their consistent presence across samples suggests a functional microbiota shaped by salting, low a_w, prolonged ripening, and variations in processing conditions among producers.

Moreover, additional clusters emerged, each consisting of a considerable number of samples and characterized by distinct microbial profiles. Cluster 8, for instance, including 17.3 % of ham samples, was markedly dominated by *S. equorum*, which reached an average relative abundance of 93.8 %, indicating a highly specialized microbial signature with limited overall diversity (58 species) (Fig. 1c, Table S3).

Interestingly, cluster 7 was composed entirely of hams matured for 18 months and was primarily characterized by *Multiple species* belonging to the genus *Tetragenococcus*, with a cumulative relative average abundance of 81.8 %, suggesting a microbial community shaped by the specific conditions associated with this stage of ripening (Fig. 1c, Table S1, S3). Its species richness (180 species) indicates a moderately complex community, possibly linked to the physicochemical properties typical of this ripening stage, such as salt concentration and a_w. At 18 months, a_w is lower than in fresher hams but not as reduced as in fully ripened products, creating favorable conditions for the proliferation of halotolerant and fermentative *Tetragenococcus* species, well adapted to the selective pressures of salt concentration and reduced moisture typical of this maturation phase (Yao et al., 2022; Link et al., 2021). Thus, the distinctive microbial profile of cluster 7 may be closely linked to the physicochemical properties typical of this specific stage of ripening.

Additionally, cluster 2, representing 12.2 % of the whole dataset, was characterized almost exclusively by *T. halophilus* as the predominant species, with a prevalence of 83 % and average relative abundance of 100 % (Fig. 1c, Table S3). The consistent detection of this species across various dry-cured ham samples further highlights its important role in the microbial ecology and maturation of these products.

Metagenomic insights into the functional potential of ham microbiota

To further explore the functional potential of the ham microbiota, shotgun metagenomic sequencing was performed on two representative samples, both of which belonged to cluster 7 (Table S4). These are 18-month-ripened hams, representing one of the earliest stages of ripening within our dataset. Cluster 7 was selected explicitly because these samples retain a higher microbial load compared to more extensively ripened products, resulting in a greater total DNA yield and thereby improving the reliability and depth of metagenomic analyses. Additionally, the moderate complexity of the microbial community in this cluster allows a robust functional profiling, capturing key metabolic activities of ham-associated microbiota at this ripening stage when communities are diverse and metabolically active. Reads were annotated against the Rhea database, and only functions with relative abundances greater than 0.1 % were considered, focusing on those known to contribute to organoleptic features, including proteolysis, lipid metabolism, and the biosynthesis of volatile compounds. Functional annotation revealed a broad spectrum of metabolic pathways relevant to the development of sensory qualities (Table S5) (Fig. 2). Carbohydrate-related functions were linked to the production of glycerol, lactic acid, furfural derivatives, and vanillin, compounds that can influence sweetness, acidity, and the emergence of caramel- or vanilla-like notes (Li et al., 2025b) (Table S5) (Fig. 2). Amino acid degradation pathways support the generation of branched-chain alcohols and acids, including 2-methylbutanol, isovaleric acid, and 2-methylbutanoic acid, which are commonly reported in the literature as contributors to fruity, cheesy, or matured meat aromas (Table S5) (Fig. 2). Additionally, the catabolism of branched-chain amino acids such as leucine and isoleucine produces aldehydes like 3-methylbutanal and 2-methylbutanal, which are recognized as key aroma compounds contributing to the characteristic cured and malty notes of dry-cured hams (Toldra and

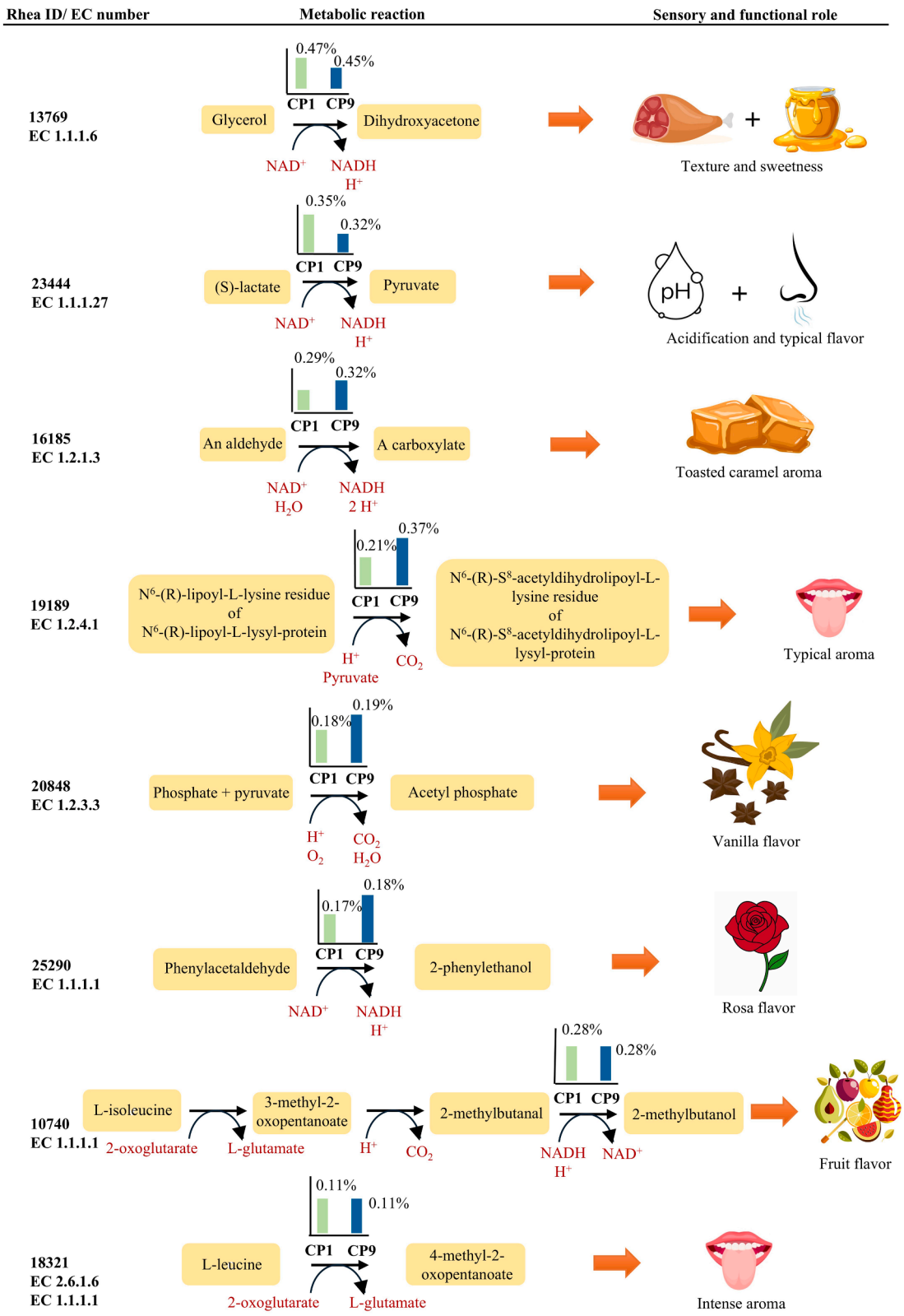


Fig. 2. Microbial functional pathways associated with organoleptic properties in dry-cured hams. Schematic representation of some metabolic pathways identified in two 18-month-ripened ham samples from cluster 7, as reconstructed through shotgun metagenomic sequencing and functional annotation against the Rhea database. The left column reports the Rhea reaction code and the corresponding EC number; the central column shows the chemical reaction; the right column indicates the final product with its associated sensory (organoleptic) role. Highlighted pathways include carbohydrate metabolism, amino acid degradation, and lipid conversion, all of which contribute to the biosynthesis of compounds associated with sweetness, acidity, fruity, cheesy, or mature-meat notes. More detailed information on the identified pathways and their relative abundances is provided in Supplementary Table 5.

Flores, 1998; Skene et al., 2008; Bai et al., 2019). In parallel, lipid metabolism contributed to the formation of aliphatic alcohols, conferring sweet and fruity nuances (Lee et al., 2025; Shahidi and Hossain, 2022). Although each pathway was detected at relatively low abundance (with a relative abundance ranging from 0.47 % to 0.11 %), their combined presence indicates that ham-associated microbes harbor an enzymatic potential well-suited to shaping flavor and texture through multiple and complementary pathways (Table S5) (Fig. 2). In addition to these sensory-relevant processes, functions related to nucleic acid metabolism, protein phosphorylation, and cell wall biosynthesis were consistently detected (Table S5) (Fig. 2). While these activities mainly reflect essential cellular processes, their persistence highlights the functional stability of the ham microbiota, supporting its ability to sustain growth and adaptation under ripening conditions. Together, these findings underscore that the microbial community of dry-cured ham is not only taxonomically diverse but also functionally equipped to produce key metabolites that contribute to the distinctive sensory properties of the final product. However, it should be noted that these microbial activities occur in parallel with those of endogenous (muscle-derived) enzymes, which also play an important role in proteolysis and lipolysis during processing. While the specific contributions of endogenous and microbial enzymes cannot be quantitatively distinguished in the present study, previous research has shown that endogenous enzymes dominate the early and intermediate stages, while

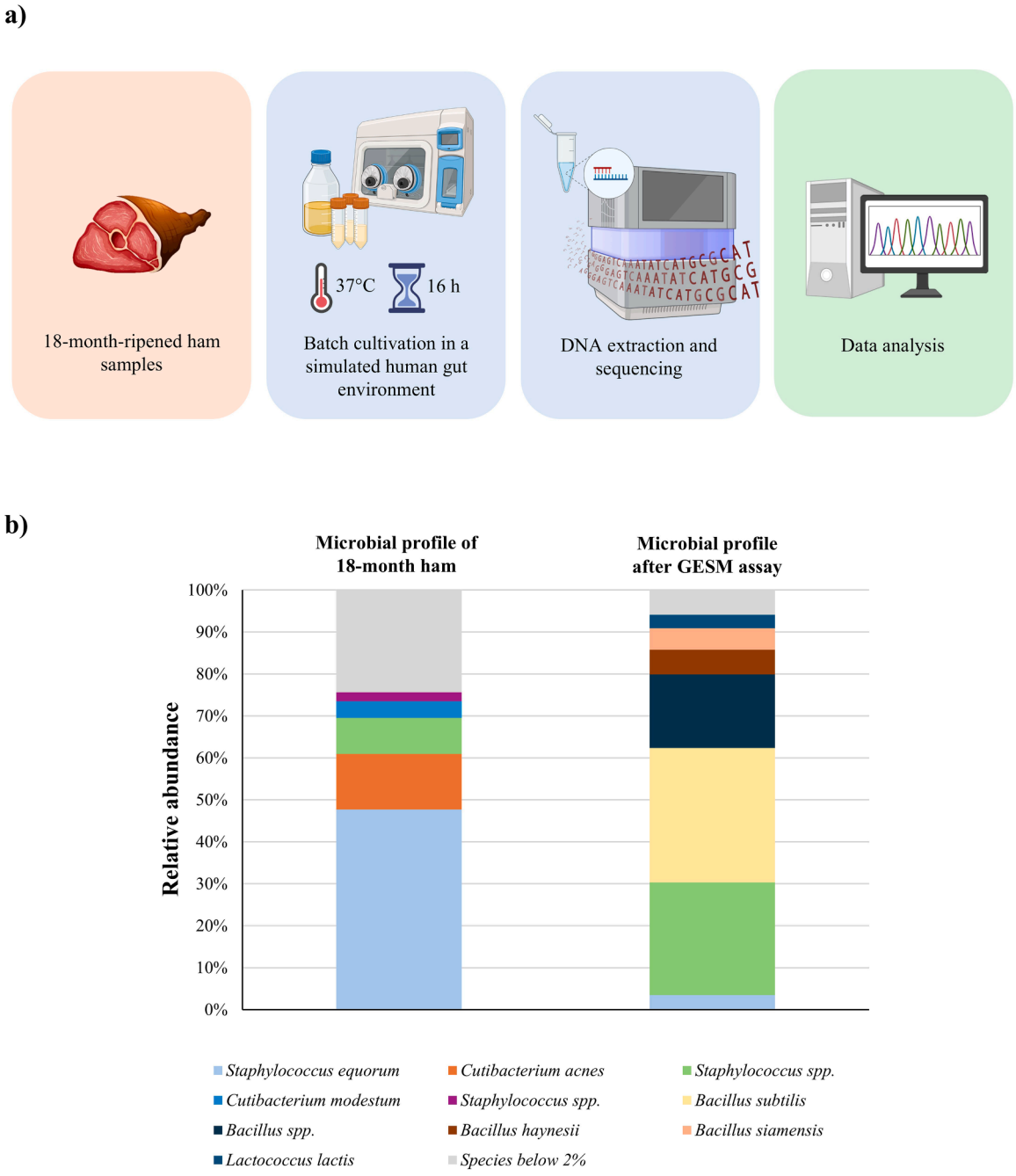


Fig. 3. Microbial analysis workflow and taxonomic profile of dry-cured ham after cultivation in GESM. Panel (a) illustrates the experimental workflow designed to explore the taxonomic shifts occurring after batch cultivation of 18-month-ripened dry-cured ham samples in a simulated intestinal environment (GESM). Panel (b) shows bar plots depicting the relative abundance of the microbial community before and after incubation in GESM, calculated as the average of three biological replicates. Only bacterial taxa with a relative abundance of ≥2 % in at least one condition are displayed.

microbial enzymes play an increasing role during the late ripening phase, particularly in surface and subsurface portions (Alapont et al., 2015; Toldra and Flores, 1998). Therefore, the metabolites observed likely result from the combined and sequential action of both enzymatic sources. Lastly, although metagenomic sequencing was performed on two representative samples to ensure high-quality and deeply resolved functional data, we acknowledge that this limited subset may not fully capture the functional diversity across all samples. Future studies, including a larger number of metagenomic datasets, will help confirm and extend these findings.

Exploration of the microbial community derived from dry-cured ham during batch growth in a simulated human gut environment

Microorganisms naturally associated with food products can reach the human gastrointestinal tract and depending on their ecological adaptability, may transiently persist or even influence gut microbial communities. To investigate the ability of dry-cured ham microbiota to survive and grow under conditions resembling the human gut, selected ham samples were cultivated in a fecal-based medium that simulates the intestinal environment. For this assay, hams specifically selected at the 18-month ripening stage were used, as this stage is typical of traditional production and represents an intermediate ripening phase, providing a suitable model to study the ability of ham-associated microorganisms to survive and adapt. At 18 months, the microbial community is expected to be more diverse and metabolically active compared to 24-month-ripened products, where lower a_w and stronger selective pressures result in a more limited microbiota. A total of three biological replicates of 18-month-ripened hams were incubated for 16 h in batch culture using a gut environment-simulating medium (referred to as GESM). This medium was chosen for its proven capacity to replicate the complex nutrient landscape of the human colon and has been widely employed in previous studies investigating microbial dynamics under intestinal conditions (Macfarlane et al., 1998; Haboubi et al., 1988; Milani et al., 2025). Both the original samples and the cultures obtained after GESM incubation were subjected to DNA extraction and shallow shotgun metagenomic sequencing (Table S6).

A marked reshaping of the microbial composition was observed after GESM cultivation compared to the original ham microbiota (Fig. 3b, Table S6). Intriguingly, the dominant bacterial species in 18-month-ripened hams, primarily belonging to the genus *Staphylococcus* (with an average relative abundance of 58.3 %), showed a considerable reduction in their presence when hams were cultivated in GESM (Fig. 3b, Table S6). Conversely, *Bacillus* species, initially detected in the original ham samples at very low levels, grew markedly in GESM, reaching an average relative abundance of 60.4 %, while *Staphylococcus* species decreased to 30.2 % (Fig. 3b, Table S6).

S. equorum dominates the microbial community in the original ham-based matrix, likely due to its adaptation to the high-salt and low a_w conditions typical of dry-cured ham and its frequent association with traditional production environments, suggesting a central role in shaping the microbiota (Martinez-Onandi et al., 2019; Jeong et al., 2017). Its metabolic activities, including proteolysis and lipolysis, may contribute to the development of flavor and texture, while its dominance promotes a relatively stable and resilient microbial community during ripening. Initially, six species had a relative abundance above 1 %, together representing 76.5 % of the total community (Fig. 3b, Table S6). After batch cultivation, eight species exceeded this threshold, representing 95.8 % of the community (Fig. 3b, Table S6). This moderate increase suggests that the gut-like environment promotes the proliferation of previously low-abundance taxa while favoring a more balance distribution among the dominant members. In other words, exposure to nutrient-rich, dynamic conditions potentially allows a broader subset of microbes to thrive, enhancing their representation in the community. A similar trend has been observed in studies on cheese microbiota, where exposure to intestinal conditions led to a selective reshaping of the microbial community, with specific food-associated taxa being favored (Milani et al., 2025; Milani et al., 2019). This observation supports the notion that the intestinal environment exerts a selective pressure on food-derived bacteria, shaping their persistence and potential interactions within the gut ecosystem. Following incubation, *Bacillus subtilis* (32.0 %), *Staphylococcus* spp. (26.8 %) and other *Bacillus* species (*B. haynesii, B. siamensis*) became dominant, while *S. equorum* decreased to 3.5 % (Fig. 3b, Table S6). Notably, the ability of *Bacillus* spp. to persist under simulated gut conditions should be interpreted with caution, as this genus includes both species with recognized probiotic potential and others occasionally associated with opportunistic behavior. Therefore, while our findings suggest that *Bacillus* spp. exhibit a high tolerance to simulated intestinal environments, the results should not provide evidence of probiotic functionality or pathogenic potential. *Lactococcus lactis*, a species often associated with beneficial metabolic activities, also emerged at 3.2 % relative abundance (Fig. 3b, Table S6). After batch cultivation, the number of species with relative abundance above 1 % increased to eight, collectively accounting for 95.8 % of the community (Fig. 3b, Table S6). These changes reflect the influence of a nutrient-rich, dynamic gut-like environment, which favors the proliferation of previously low-abundance taxa and leads to a more even distribution among dominant and subdominant members.

This striking shift highlights how microbial taxa that are only marginally represented in the food matrix can outcompete dominant ham-associated microbes when exposed to the nutrient-rich and dynamic environment that mimics the human gut.

Conclusions

Foodborne microbiomes are increasingly recognized as contributors to the sensory qualities of fermented foods and potential modulators of the human gut ecosystem. In this study, we provided a comprehensive characterization of the microbiota associated with traditional Italian dry-cured hams through high-throughput sequencing and metagenomic approaches. A conserved microbial core dominated by *S. equorum* was identified, together with diverse accessory taxa whose prevalence and abundance varied according to geographical origin, maturation stage, and processing practices. Cluster analyses highlighted the complexity of these microbial ecosystems, which rather than aligning strictly with product type or ripening duration, appeared to be shaped by the combined influence of producer practices, raw material characteristics and local curing microenvironmental conditions. Moreover, functional profiling revealed that ham-associated microbiota harbors metabolic pathways involved in amino acid, carbohydrate, and lipid catabolism, which contribute to flavor and texture development. Interestingly, cultivation in a simulated intestinal environment demonstrated that low-abundance microbial taxa can survive and proliferate under gut-like conditions suggesting a potential capacity for transient persistence.

Taken together, our findings underscore the dual role of dry-cured ham microbiota, which contributes to the organoleptic features of the product and include microorganisms able to grow in an *in vitro* gut resembling model. Increasing evidence indicates that microorganisms originating from foods and water can transiently colonize the human gut digestive tract (Milani et al., 2019; Milani et al., 2025; Lugli et al., 2022). Although our study does not demonstrate that ham microbes can colonize the human gut, it provides preliminary *in vitro* evidence that some ham-associated microbes can withstand simulated gastrointestinal conditions, an aspect that may warrant further investigation. Future studies, integrating metabolomics and *in vivo* models, will be crucial in assessing the persistence, functionality, and potential health impacts of these foodborne microbes.

Funding

Project funded under the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.3 - Call for tender No 341

of 15 March 2022 of Italian Ministry of University and Research funded by the European Union – NextGenerationEU; Award Number: Project code PE00000003, Concession Decree No 1550 of 11 October 2022 adopted by the Italian Ministry of University and Research, CUP D93C22000890001, Project title "ON Foods - Research and innovation network on food and nutrition Sustainability, Safety and Security – Working ON Foods."

F.T. is founded by Piano di Sviluppo e Coesione of the Italian Ministry of Health 2014–2020 (Grant Number T5-AN-11).

Authors' contributions

GL retrieved and processed ham samples, performed all experiments and wrote the manuscript; CT managed the metadata and performed the bioinformatics analyses; LA, SA and AV sequenced the samples; MV supervised the project and designed the study; CM validated the bioinformatics analyses and edited the manuscript; FT supervised the project and edited the manuscript.

Declaration of competing interest

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

Acknowledgments

We gratefully thank Consorzio del Prosciutto di Parma for providing the ham samples used in this study. We acknowledge GenProbio Srl for the financial support of the Laboratory of Probiogenomics and the PNRR ONFOODS funding. Figures were prepared with BioRender (https://biorender.com) and images from Freepik (https://www.freepik.com), both used in accordance with their licensing agreements.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.crmicr.2025.100516.

References

ALAPONT, C., MARTINEZ-CULEBRAS, P.V., LOPEZ-MENDOZA, M.C., 2015. Determination of lipolytic and proteolytic activities of mycoflora isolated from dry-cured teruel ham. J. Food Sci. Technol. 52, 5250–5256.

BAI, J., YU, Q., SUN, X., XIAO, H., WANG, K., SUN, F., SUI, Q., 2019. Hydrocephalus due to idiopathic fourth ventricle outflow obstruction. J. Craniofac. Surg. 30, e397–e400.

BANSAL, P., MORGAT, A., AXELSEN, K.B., MUTHUKRISHNAN, V., COUDERT, E., AIMO, L., HYKA-NOUSPIKEL, N., GASTEIGER, E., KERHORNOU, A., NETO, T.B., POZZATO, M., BLATTER, M.C., IGNATCHENKO, A., REDASCHI, N., BRIDGE, A., 2022. Rhea, the reaction knowledgebase in 2022. Nucleic. Acids. Res. 50, D693–D700.

BIBBO, S., IANIRO, G., GIORGIO, V., SCALDAFERRI, F., MASUCCI, L., GASBARRINI, A., CAMMAROTA, G., 2016. The role of diet on gut microbiota composition. Eur. Rev. Med. Pharmacol. Sci. 20, 4742–4749.

CERRATO, A., AITA, S.E., CAPRIOTTI, A.L., CAVALIERE, C., MONTONE, A.M.I., MONTONE, C.M., LAGANA, A., 2022. Investigating the short peptidome profile of Italian dry-cured ham at different processing times by high-resolution mass spectrometry and chemometrics. Int. J. Mol. Sci. 23.

DE FILIPPIS, F., LA STORIA, A., STELLATO, G., GATTI, M., ERCOLINI, D., 2014. A selected core microbiome drives the early stages of three popular italian cheese manufactures. PLoS. One 9, e89680.

FONTANA, F., LONGHI, G., ALESSANDRI, G., LUGLI, G.A., MANCABELLI, L., TARRACCHINI, C., VIAPPIANI, A., ANZALONE, R., VENTURA, M., TURRONI, F., MILANI, C., 2023. Multifactorial microvariability of the Italian raw milk cheese microbiota and implication for current regulatory scheme. mSystems 8, e0106822.

FONTANA, F., LONGHI, G., CARLI, E., ALESSANDRI, G., MANCABELLI, L., LUGLI, G.A., TARRACCHINI, C., VIAPPIANI, A., ANZALONE, R., TURRONI, F., MILANI, C., VENTURA, M., 2024. Revealing the genetic traits of the foodborne microbial genus hafnia: implications for the human gut microbiome. Environ. Microbiol. 26, e16626.

GUTSCHE, K.A., TRAN, T.B., VOGEL, R.F., 2012. Production of volatile compounds by Lactobacillus sakei from branched chain alpha-keto acids. Food Microbiol. 29, 224–228.

HABOUBI, N.Y., BAKER, M.A., GYDE, O.H., SMALL, N.A., HABOUBI, N., 1988. Zinc supplementation and erythropoiesis in the elderly. J. Clin. Pathol. 41, 706.

HARKOUSS, R., MIRADE, P.S., GATELLIER, P., 2012. Development of a rapid, specific and efficient procedure for the determination of proteolytic activity in dry-cured ham: definition of a new proteolysis index. Meat. Sci. 92, 84–88.

JEONG, D.W., HEO, S., RYU, S., BLOM, J., LEE, J.H., 2017. Genomic insights into the virulence and salt tolerance of Staphylococcus equorum. Sci. Rep. 7, 5383.

KOLBECK, S., ABELE, M., HILGARTH, M., VOGEL, R.F., 2021. Comparative proteomics reveals the Anaerobic lifestyle of meat-spoiling Pseudomonas species. Front. Microbiol. 12, 664061.

LANDETA, G., CURIEL, J.A., CARRASCOSA, A.V., MUNOZ, R., DE LAS RIVAS, B., 2013. Characterization of coagulase-negative staphylococci isolated from Spanish dry cured meat products. Meat. Sci. 93, 387–396.

LEE, S., JO, K., PARK, M.K., CHOI, Y.S., JUNG, S., 2025. Role of lipids in beef flavor development: a review of research from the past 20 years. Food Chem. 475, 143310.

LEROY, S., LEBERT, I., CHACORNAC, J.P., CHAVANT, P., BERNARDI, T., TALON, R., 2009. Genetic diversity and biofilm formation of Staphylococcus equorum isolated from naturally fermented sausages and their manufacturing environment. Int. J. Food Microbiol. 134, 46–51.

LI, D., LIANG, Y., XIA, Q., PAN, D., DU, L., HE, J., SUN, Y., WANG, Y., WANG, W., CAO, J., ZHOU, C., 2025a. LC-MS/MS-based metabolomics and multivariate statistical analysis reveal the mechanism of yeast extracellular proteases on myofibrillar protein degradation, metabolite development and sensory characteristics improvement. Food Microbiol. 128, 104715.

LI, L., BELLOCH, C., FLORES, M., 2025b. The aroma of non-fermented and fermented dry-cured meat products: savory and toasted odors. Foods. 14.

LI, R., ZHU, L., CUI, L., ZHU, Y.G., 2022. Viral diversity and potential environmental risk in microplastic at watershed scale: evidence from metagenomic analysis of plastisphere. Environ. Int. 161, 107146.

LINK, T., VOGEL, R.F., EHRMANN, M.A., 2021. The diversity among the species tetragenococcus halophilus including new isolates from a lupine seed fermentation. BMC. Microbiol. 21, 320.

LIU, S., ZHAO, L., LI, M., ZHU, Y., LIANG, D., MA, Y., SUN, L., ZHAO, G., TU, Q., 2024. Probiotic Bacillus as fermentation agents: status, potential insights, and future perspectives. Food Chem. X. 22, 101465.

LUGLI, G.A., LONGHI, G., MANCABELLI, L., ALESSANDRI, G., TARRACCHINI, C., FONTANA, F., TURRONI, F., MILANI, C., VAN SINDEREN, D., VENTURA, M., 2022. Tap water as a natural vehicle for microorganisms shaping the human gut microbiome. Environ. Microbiol. 24, 3912–3923.

LUO, X., SHEN, Y., 2025. Comparative analysis of the microbial composition of three packaged sliced dry-cured hams from a Chinese market. Front. Microbiol. 16, 1531005.

MACFARLANE, G.T., MACFARLANE, S., GIBSON, G.R., 1998. Validation of a three-stage compound continuous culture system for investigating the effect of retention time on the ecology and metabolism of bacteria in the Human colon. Microb. Ecol. 35, 180–187.

MARTINEZ-ONANDI, N., CASTIONI, A., SAN MARTIN, E., RIVAS-CANEDO, A., NUNEZ, M., TORRIANI, S., PICON, A., 2017. Microbiota of high-pressure-processed Serrano ham investigated by culture-dependent and culture-independent methods. Int. J. Food Microbiol. 241, 298–307.

MARTINEZ-ONANDI, N., SANCHEZ, C., NUNEZ, M., PICON, A., 2019. Microbiota of Iberian dry-cured ham as influenced by chemical composition, high pressure processing and prolonged refrigerated storage. Food Microbiol. 80, 62–69.

MILANI, C., ALESSANDRI, G., MANGIFESTA, M., MANCABELLI, L., LUGLI, G.A., FONTANA, F., LONGHI, G., ANZALONE, R., VIAPPIANI, A., DURANTI, S., TURRONI, F., COSTI, R., ANNICCHIARICO, A., MORINI, A., SARLI, L., OSSIPRANDI, M.C., VAN SINDEREN, D., VENTURA, M., 2020. Untangling species-level composition of complex bacterial communities through a novel metagenomic approach. mSystems. 5.

MILANI, C., CASEY, E., LUGLI, G.A., MOORE, R., KACZOROWSKA, J., FEEHILY, C., MANGIFESTA, M., MANCABELLI, L., DURANTI, S., TURRONI, F., BOTTACINI, F., MAHONY, J., COTTER, P.D., MCAULIFFE, F.M., VAN SINDEREN, D., VENTURA, M., 2018. Tracing Mother-Infant Transmission of Bacteriophages By Means of a Novel Analytical Tool For Shotgun Metagenomic datasets: METAnnotatorX, 6. Microbiome, p. 145.

MILANI, C., DURANTI, S., NAPOLI, S., ALESSANDRI, G., MANCABELLI, L., ANZALONE, R., LONGHI, G., VIAPPIANI, A., MANGIFESTA, M., LUGLI, G.A., BERNASCONI, S., OSSIPRANDI, M.C., VAN SINDEREN, D., VENTURA, M., TURRONI, F., 2019. Colonization of the human gut by bovine bacteria present in Parmesan cheese. Nat. Commun. 10, 1286.

MILANI, C., LONGHI, G., ALESSANDRI, G., FONTANA, F., VIGLIOLI, M., TARRACCHINI, C., MANCABELLI, L., LUGLI, G.A., PETRARO, S., ARGENTINI, C., ANZALONE, R., VIAPPIANI, A., CARLI, E., VACONDIO, F., VAN SINDEREN, D., TURRONI, F., MOR, M., VENTURA, M., 2025. Functional modulation of the human gut microbiome by bacteria vehicled by cheese. Appl. Environ. Microbiol. 91, e0018025.

MILANI, C., LUGLI, G.A., FONTANA, F., MANCABELLI, L., ALESSANDRI, G., LONGHI, G., ANZALONE, R., VIAPPIANI, A., TURRONI, F., VAN SINDEREN, D., VENTURA, M., 2021. METAnnotatorX2: a comprehensive tool for deep and shallow metagenomic data set analyses. mSystems., e0058321

MU, Y., SU, W., MU, Y., JIANG, L., 2019. Combined application of high-throughput sequencing and metabolomics reveals metabolically active microorganisms during Panxian Ham processing. Front. Microbiol. 10, 3012.

PIASENTIER, E., PIZZUTTI, N., LIPPE, G., 2021. The influence of the type of dry-cured Italian PDO ham on cathepsin B activity trend during processing. Foods. 10.

PUGLIESE, C., SIRTORI, F., CALAMAI, L., FRANCI, O., 2010. The evolution of volatile compounds profile of "Toscano" dry-cured ham during ripening as revealed by SPME-GC–MS approach. J. Mass Spectrom. 45, 1056–1064.
QIN, Y., LI, W., ZHANG, W., ZHANG, B., YAO, D., ZENG, C., CAO, J., LI, L., HUANG, R., 2024. Characterization the microbial diversity and metabolites of four varieties of Dry-Cured ham in western Yunnan of China. Food Chem. X. 22, 101257.
QUEVILLON, E., SILVENTOINEN, V., PILLAI, S., HARTE, N., MULDER, N., APWEILER, R., LOPEZ, R., 2005. InterProScan: protein domains identifier. Nucleic. Acids. Res. 33, W116–W120.
REUBEN, R.C., LANGER, D., EISENHAUER, N., JURBURG, S.D., 2023. Universal drivers of cheese microbiomes. iScience 26, 105744.
ROSELLI, M., NATELLA, F., ZINNO, P., GUANTARIO, B., CANALI, R., SCHIFANO, E., DE ANGELIS, M., NIKOLOUDAKI, O., GOBBETTI, M., PEROZZI, G., DEVIRGILIIS, C., 2021. Colonization ability and impact on Human gut microbiota of foodborne microbes from traditional or probiotic-added fermented foods: a systematic review. Front. Nutr. 8, 689084.
SHAHIDI, F., HOSSAIN, A., 2022. Role of lipids in food flavor generation. Molecules. 27.
SHI, Q., WANG, X., JU, Z., LIU, B., LEI, C., WANG, H., LI, H., 2021. Technological and safety characterization of kocuria rhizophila isolates from traditional ethnic dry-cured ham of Nuodeng, Southwest China. Front. Microbiol. 12, 761019.
SKENE, C.D., DOIDGE, C., SUTTON, P., 2008. Evaluation of ISCOMATRIX and ISCOM vaccines for immunisation against Helicobacter pylori. Vaccine 26, 3880–3884.
SMIT, B.A., ENGELS, W.J., WOUTERS, J.T., SMIT, G., 2004. Diversity of L-leucine catabolism in various microorganisms involved in dairy fermentations, and identification of the rate-controlling step in the formation of the potent flavour component 3-methylbutanal. Appl. Microbiol. Biotechnol. 64, 396–402.
STAVROPOULOU, D.A., DE MAERE, H., BERARDO, A., JANSSENS, B., FILIPPOU, P., DE VUYST, L., DE SMET, S., LEROY, F., 2018. Species pervasiveness within the group of coagulase-negative Staphylococci associated with meat fermentation is modulated by pH. Front. Microbiol. 9, 2232.
TAMANG, J.P., WATANABE, K., HOLZAPFEL, W.H., 2016. Review: diversity of microorganisms in global fermented foods and beverages. Front. Microbiol. 7, 377.
TOLDRA, F., FLORES, M., 1998. The role of muscle proteases and lipases in flavor development during the processing of dry-cured ham. Crit. Rev. Food Sci. Nutr. 38, 331–352.
TOLDRA, F., GALLEGO, M., REIG, M., ARISTOY, M.C., MORA, L., 2020. Bioactive peptides generated in the processing of dry-cured ham. Food Chem. 321, 126689.
TOLEDANO, A., JORDANO, R., LOPEZ, C., MEDINA, L.M., 2011. Proteolytic activity of lactic acid bacteria strains and fungal biota for potential use as starter cultures in dry-cured ham. J. Food Prot. 74, 826–829.
TOSCANO, A., GIANNUZZI, D., MALGWI, I.H., DEB, S., BROCCANELLO, C., SQUARTINI, A., STEVANATO, P., CECCHINATO, A., GALLO, L., SCHIAVON, S., 2024. Characterization of dry-cured ham microbiota at 12 months of seasoning obtained from different rearing strategies using 16S rRNA profiling. Food Microbiol. 122, 104558.
UMU, O.C.O., RUDI, K., DIEP, D.B., 2017. Modulation of the gut microbiota by prebiotic fibres and bacteriocins. Microb. Ecol. Health Dis. 28, 1348886.
WANG, X., DING, Y., XIA, Q., WANG, Y., SUN, Y., PAN, D., CAO, J., ZHOU, C., 2025. The effects of yeast fermentation on taste substance evolution and sensory development of dry-cured ham: the insights of peptidomics and metabolomics. Food Chem. X. 31, 103073.
WASTYK, H.C., FRAGIADAKIS, G.K., PERELMAN, D., DAHAN, D., MERRILL, B.D., YU, F. B., TOPF, M., GONZALEZ, C.G., VAN TREUREN, W., HAN, S., ROBINSON, J.L., ELIAS, J.E., SONNENBURG, E.D., GARDNER, C.D., SONNENBURG, J.L., 2021. Gut-microbiota-targeted diets modulate human immune status. Cell 184, 4137–4153 e14.
YAO, S., HAO, L., ZHOU, R., JIN, Y., HUANG, J., WU, C., 2022. Formation of biofilm by tetragenococcus halophilus benefited stress tolerance and anti-biofilm activity against S. aureus and *S. typhimurium*. Front. Microbiol. 13, 819302.
ZHANG, L., TUOLIKEN, H., LI, J., GAO, H., 2025a. Diet, gut microbiota, and health: a review. Food Sci. Biotechnol. 34, 2087–2099.
ZHANG, T., WANG, Y., ZHU, J., CHEN, C., JIANG, T., FANG, S., LIU, H., TONG, H., XIA, Q., SUN, Y., PAN, D., CAO, J., ZHOU, C., 2025b. TMT-labelled quantitative proteomics reveals the mechanism of rhodotorula mucilaginosa on proteolysis of dry-cured ham: structural protein degradation, amino acid release and taste improvement. Food Chem. 472, 142991.