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Beyond metagenomics: culturomics uncovers aerobic and facultative anaerobic bacterial diversity in the camel gut

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Abstract

While metagenomics has transformed our view of microbial ecosystems, culture-based methods remain indispensable for accessing microbial functionality and biotechnological potential. In this study, we applied a culturomics strategy to explore the diversity, abundance, and distribution of culturable aerobic and facultative anaerobic bacteria along the gastrointestinal tract of dromedary camels (*Camelus dromedarius*) grazing on pristine desert flora. Using six culture media—including modified YCFA formulations—we isolated 97 bacterial species across 42 genera, 31 families, and four phyla: Firmicutes, Proteobacteria, Actinomycetota, and Bacteroidota. Strikingly, 88.6% of this diversity was recovered using YCFA-based media, and four candidate novel species were identified. The rumen harbored the most diverse and Gram-positive–dominated community, whereas the small intestine was enriched with Gram-negative taxa, many with pathogenic potential. These findings highlight the camel's unique physiological adaptation to extreme arid environments, characterized by efficient fiber degradation under nutrient- and water-limited conditions and the presence of stress-tolerant gut microbes capable of resisting acidic and osmotic challenges. Overall, this study establishes a foundational understanding of the camel gut microbiota and underscores the complementary power of culture-dependent methods to metagenomics. Future integration with anaerobic culturing and multi-omics analyses will further unveil the ecological and biotechnological potential of desert-adapted microbial life.

Keywords Camel microbiota, Culturable bacteria, Culturomics, Bacterial diversity, Gastrointestinal ecology, Desert-adapted microbes

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Introduction

The gastrointestinal tract (GIT) of ruminants harbors a complex and dynamic microbial ecosystem that underpins digestion, immune development, and adaptation to environmental stressors [1]. Among these animals, the dromedary camel (*Camelus dromedarius*) is a remarkable desert-adapted species capable of extracting nutrients from fibrous, low-quality forage under conditions of extreme heat, drought, and dietary toxicity. Despite their ecological and agricultural importance, the microbial communities supporting these physiological adaptations remain insufficiently characterized—particularly through culture-based approaches [2, 3].

Advances in high-throughput sequencing have revolutionized microbiome research, revealing microbial diversity with unprecedented resolution. However, culture-independent methods, although powerful, frequently overlook functionally significant taxa or novel microbial lineages due to database biases and the inability to provide live isolates for downstream studies [4]. Culturomics—a systematic, high-throughput revival of culture-based microbiology—offers a complementary strategy that enables the recovery of viable and metabolically diverse microbes, including rare and previously uncharacterized species [5, 6]. While culturomics has been widely applied to the human gut microbiota, its use in non-model or desert-adapted animals remains extremely limited [7, 8].

In this study, we applied a culturomics framework to characterize aerobic and facultative anaerobic bacterial communities across three major compartments of the camel GIT: the rumen, abomasum, and small intestine. Using selective and modified culture media including optimized YCFA (Yeast extract-Casitone- fatty acids medium) formulations under controlled atmospheric conditions, we aimed to maximize the recovery of diverse cultivable taxa. Our findings provide new insights into the taxonomic structure and potential ecological functions of the camel gut microbiota and highlight the value of culturomics as a powerful complement to metagenomics. This work establishes a foundation for future studies integrating anaerobic cultivation and multi-omics approaches to advance our understanding of microbial resilience, adaptation, and innovation in arid ecosystems.

Methods

Study areas and sampling

Rumen digesta samples were collected from one female and two male single-humped camels (*Camelus dromedarius*) aged between 2 and 5 years. Sampling was conducted in Qom, Iran (latitude 34°43′6.26″ N, longitude 50°41′56.54″ E), a region characterized by a semi-arid climate with annual rainfall below 100 mm and an average summer temperature of approximately 41.5 °C.

The camels were selected based on their natural grazing behavior in a native semi-arid environment dominated by salt-tolerant plant species such as *Tamarix*, *Salsola*, *Alhagi camelorum*, *Aristida*, and *Cenchrus*. The animals were not manually fed and received no supplementary diet. This natural feeding regime was considered essential to ensure a representative microbial diversity in the gastrointestinal tract (GIT), given the strong influence of diet on gut microbiota composition.

Gastrointestinal contents—including both liquid and solid fractions—from the rumen, abomasum, and small intestine were collected immediately post-slaughter at a commercial abattoir. All samples were transported on ice and stored at 4 °C until further processing. The study was conducted in accordance with the national guidelines for laboratory animal care and approved by the Iranian Veterinary Organization.

Culture media preparation

The DSMZ medium 1611 (Yeast extract–Casitone–Fatty acid, YCFA) was used as the basal medium for the isolation of rumen bacteria. The original formulation contained 0.005 g/mL glucose (Supplementary Table S1). To broaden the spectrum of recoverable taxa, the medium was modified to YCFA+2 G by reducing the glucose concentration to 0.002 g/mL and supplementing it with 0.002 g/mL each of maltose and cellobiose. To mimic the bile salt environment characteristic of the rumen, sodium taurocholate (0.1%) was incorporated into both YCFA and YCFA+2 G formulations, yielding YCFA+S and YCFA+2GS media, respectively [1, 9]. Sodium taurocholate is known to promote the growth of bile-tolerant bacteria that are abundant in the rumen ecosystem. Nutrient Agar (NA) and Tryptic Soy Agar (TSA) were used as control media. For long-term preservation, bacterial isolates were maintained in the corresponding broth medium containing 25% (v/v) glycerol and stored at – 80 °C.

Culturing and isolation

Gastrointestinal contents were homogenized in 0.1 M phosphate-buffered saline (PBS, pH 7.0). From the resulting suspension, 100 µL of the initial sample and serial dilutions up to 10 [7] were spread onto the prepared culture media under both aerobic and facultative anaerobic conditions at 37 °C (with 5% CO_2 for facultative anaerobes) [1]. For each dilution, three independent replicates were plated to maximize the likelihood of successful microbial recovery under varying growth conditions.

Additionally, tissue samples from the GIT compartments were washed with sterile PBS, resuspended, and processed following the same procedure. Colonies were selected after 72 hours of incubation, re-streaked on the corresponding medium to ensure purity, and preserved in glycerol stocks at – 80 °C for subsequent analyses.

DNA extraction, PCR amplification and sequencing of the 16S rRNA gene

Genomic DNA was extracted from each isolate using a heat lysis method (100 °C for 10 min) [10]. The 16S rRNA gene was amplified using universal bacterial primers 27F and 1492 R [11]. PCR amplification was performed under the following conditions: initial denaturation at 95 °C for 10 min; 40 cycles of denaturation at 95 °C for 60 s, annealing at 58 °C for 60 s, and extension at 72 °C for 90 s; followed by a final extension at 72 °C for 10 min. The amplified products were purified and subjected to Sanger sequencing (Macrogen Inc., South Korea). Taxonomic identification of isolates was performed based on 16S rRNA gene sequence analysis using the EzTaxon database. Isolates showing ≥ 98.65% sequence similarity to reference sequences were considered to belong to the same species, following established thresholds in bacterial taxonomy [12].

Phylogenetic analysis

The 16S rRNA gene sequences were aligned using the ClustalW algorithm implemented in MEGA version 7.0. Phylogenetic trees were constructed using the neighbor-joining method, and the reliability of the branching was assessed through 1000 bootstrap replicates. The Kimura two-parameter (K2P) model was applied as the best-fit nucleotide substitution model [13].

Diversity and similarity determination

Statistical significance among various gut segments and media cultures was calculated using the Kruskal–Wallis test. Beta diversity analysis, permutational multivariate analysis of variance (PERMANOVA), and principal coordinates analysis (PCoA) based on Bray–Curtis dissimilarity were performed in R v4.5.2 using the *vegan*, *ggplot2*, and *heatmap* packages. Furthermore, microbial richness and Shannon effective numbers were calculated and visualized using GraphPad Prism v9.4.1.

Heatmaps were generated using ClustVis (https://biit.cs.ut.ee/clustvis/) based on genus-level relative abundance data [14]. Prior to visualization, data were log-transformed and standardized using Z-score normalization across compartments or media, such that color gradients represent values below or above the mean abundance of each genus.

The visualization of shared and unique bacterial species between the rumen, abomasum, and small intestine was performed using InteractiVenn (https://www.interactivenn.net/index2.html) [15].

Data availability

The 16S rRNA gene sequences obtained from all bacterial isolates retrieved in this study have been deposited in NCBI GenBank as Dataset 1. Corresponding accession numbers are provided in Supplementary Table S2.

Results

Diversity of culturable bacteria identified in the gastrointestinal tract

Using a culturomics-based approach, we isolated a total of 1105 bacterial colonies from the rumen (pH 6.0–6.4), abomasum (pH 3.5–4.0), and small intestine (pH 6.7–8.0) of three healthy adult camels. Colonies were selected based on morphological diversity—size, shape, texture, pigmentation, and margins—and were identified by 16S rRNA gene sequencing to ensure phylogenetic breadth. From this collection, 472 representative strains were deposited in GenBank (Supplementary Table S2), encompassing 97 bacterial species across 42 genera, 31 families, and 15 orders. These taxa belonged to four phyla: *Firmicutes* (56.35%), *Proteobacteria* (37.79%), *Actinomycetota* (5.64%), and *Bacteroidota* (0.22%).

Firmicutes and *Proteobacteria* were dominant across all gastrointestinal compartments; however, their relative abundances differed by region: *Firmicutes* predominated in the rumen and abomasum, while *Proteobacteria* were more abundant in the small intestine (Fig. 1A). *Actinomycetota* and *Bacteroidota* were less frequently recovered. Notably, *Bacteroidota* were detected only in the rumen and not in the abomasum or small intestine. *Actinomycetota* accounted for approximately 5% of abomasal isolates and about 8% in the rumen. In contrast, *Firmicutes* constituted only ~7% of the small intestinal isolates. At the order level, *Lactobacillales*, *Bacillales*, and *Enterobacterales* were the most prevalent across compartments (Supplementary Fig. S1).

The relative abundances of bacterial families isolated from the total culture collection obtained via culturomics across the camel gastrointestinal tract were examined. Among the 16 identified families, Lactobacillaceae was the most abundant (14.29%), followed by Bacillaceae, Enterobacteriaceae, and Enterococcaceae (each 9.89%). Streptococcaceae, Staphylococcaceae, and Planococcaceae were also relatively prevalent, with abundances of 7.69%, 5.49%, and 4.39%, respectively. Other families, including Microbacteriaceae, Micrococcaceae, Moraxellaceae, Pseudomonadaceae, Brevibacteriaceae, Carnobacteriaceae, Leuconostocaceae, and Paenibacillaceae, contributed smaller fractions (2.19–3.29%), while a combined "Others" category accounted for 17.58% of the total isolates. These results highlight the dominance of Gram-positive families in the culturable camel gut microbiota, while also reflecting the presence of diverse Gram-negative taxa (Fig. 2).

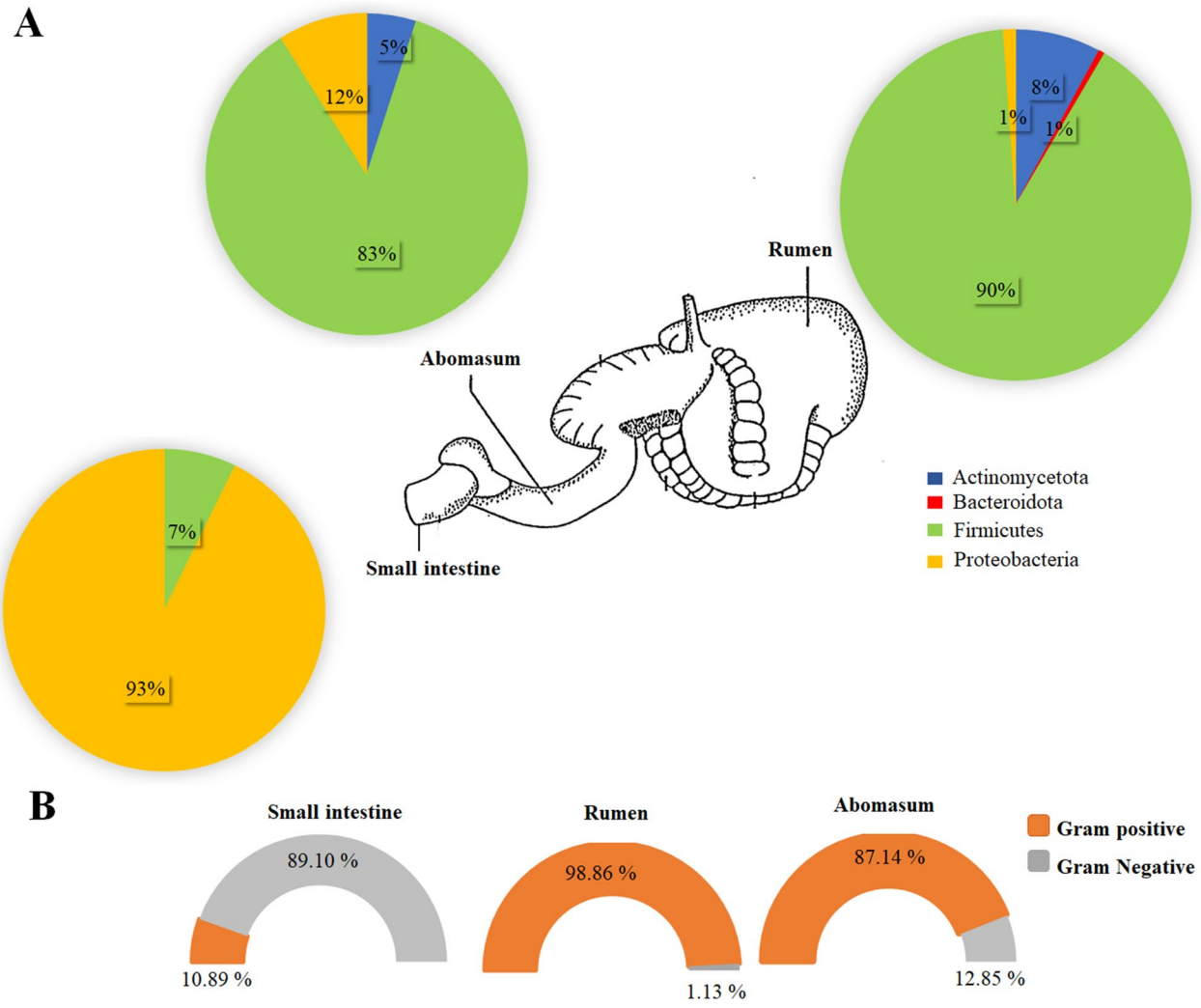

Fig. 1 Abundance of bacterial phyla retrieved by culturomics. **A**: Comparison of the percentage of bacterial phyla isolated from different compartments of the camel GIT, including the rumen, abomasum, and small intestine. **B**: Distribution of Gram-positive and Gram-negative bacteria in each GIT compartment under aerobic and facultative anaerobic conditions

Gram-staining classification revealed a clear spatial trend: Gram-positive bacteria dominated the rumen and abomasum, whereas Gram-negative bacteria predominated in the small intestine (Fig. 1B).

Distinct differences were also observed between bacterial communities associated with the tissue surface and those present in the luminal content (Fig. 3). In the rumen, abomasum, and small intestine, 64%, 91%, and 66% of the isolated taxa, respectively, were recovered from floated gastrointestinal content (Fig. 3A).

In the rumen, *Actinomycetota* showed similar abundance across both compartments, while *Firmicutes* were underrepresented on the tissue surface, and *Proteobacteria* and *Bacteroidota* were absent altogether. In the abomasum, *Actinomycetota* represented less than 20% of isolates across both compartments, while *Firmicutes* dominated the luminal microbiota, accounting for nearly 80% of isolates. Notably, *Proteobacteria* were exclusively identified in luminal samples, highlighting compartment-specific bacterial distribution. In the small intestine, both *Firmicutes* and *Proteobacteria* were more abundant in luminal content than on the tissue surface (Fig. 3B).

A subset of bacterial species was shared between the tissue and luminal compartments (Fig. 3C). In the abomasum, Arthrobacter citreus and *Enterococcus durans* were common to both niches. The rumen exhibited the highest number of shared taxa, including *Arthrobacter citreus*, *Bacillus altitudinis*, Bacillus licheniformis, Bacillus zhangzhouensis, Enterococcus durans, Enterococcus hirae, Lactobacillus coryniformis, Lactobacillus curvatus, Lactobacillus plantarum, and Lactobacillus sakei. In the small intestine, shared species included Escherichia fergusonii, Hafnia alvei, Pantoea dispersa,

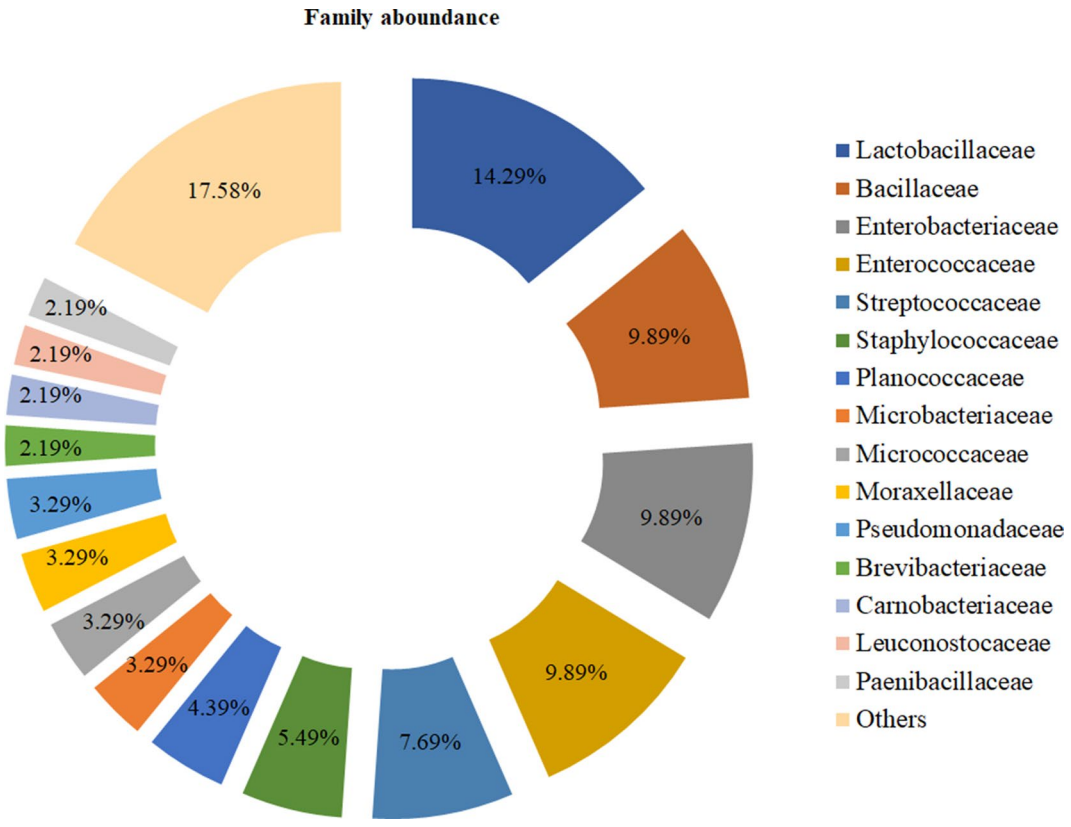

Fig. 2 Abundance of bacterial families retrieved in the culturable collection via culturomics from the rumen, abomasum, and small intestine of the camel

Shigella dysenteriae, Shigella flexneri, Shigella sonnei, and Enterococcus durans.

Microbial composition and distribution across the GIT

The bacterial community composition exhibited pronounced compartmentalization across the three gastrointestinal tract (GIT) sections. In the rumen, the community was strongly dominated by *Lactobacillaceae* (67.55%), followed by *Bacillaceae* (3.87%), *Micrococcaceae* (3.39%), and *Enterococcaceae* (2.42%). Several low-abundance families, including *Streptomycetaceae*, *Staphylococcaceae*, and *Microbacteriaceae*, were detected exclusively in this compartment, indicating higher family-level diversity and potential functional complexity.

In the abomasum, *Lactobacillaceae* remained the predominant family (65.84%), accompanied by relatively higher proportions of *Enterococcaceae* (10.40%) and *Enterobacteriaceae* (6.93%). Families such as *Planococcaceae* (2.48%) and *Pseudomonadaceae* (1.49%) were uniquely present, further emphasizing compartment-specific microbial patterns.

The small intestine displayed a markedly distinct profile, overwhelmingly dominated by *Enterobacteriaceae* (92.44%) and Enterococcaceae (6.98%), while *Lactobacillaceae* declined to 0.29%. Notably, *Hafniaceae* (0.29%) were also exclusively recovered from this section (Supplementary Table S3).

Overall, the rumen and abomasum shared broadly similar profiles, characterized by dominant *Lactobacillaceae* and relatively high family-level diversity. In contrast, the small intestine harbored a highly specialized microbial community, suggesting functional differentiation along the camel GIT. These compartment-specific patterns provide insights into niche specialization and potential host–microbe interactions in ruminants, highlighting the ecological complexity of the camel gastrointestinal microbiota.

At the genus level, *Lactobacillus, Shigella, Escherichia, Pediococcus*, and *Enterococcus* were the most abundant across all gastrointestinal compartments (Fig. 4A; Supplementary Table S4). Diversity at this taxonomic level was highest in the rumen, which harbored a broader range of taxa, including compartment-specific genera such as Acinetobacter, Bacillus, and Pseudomonas, whereas Enterococcus and Streptococcus were more prevalent in the abomasum (Fig. 4B).

At the species level, dominant taxa included *Lactobacillus sakei, Shigella flexneri, Pediococcus pentosaceus*, and *Escherichia fergusonii* (Supplementary Table S5). Distribution patterns exhibited clear compartment-specificity, with some species confined to individual gastrointestinal

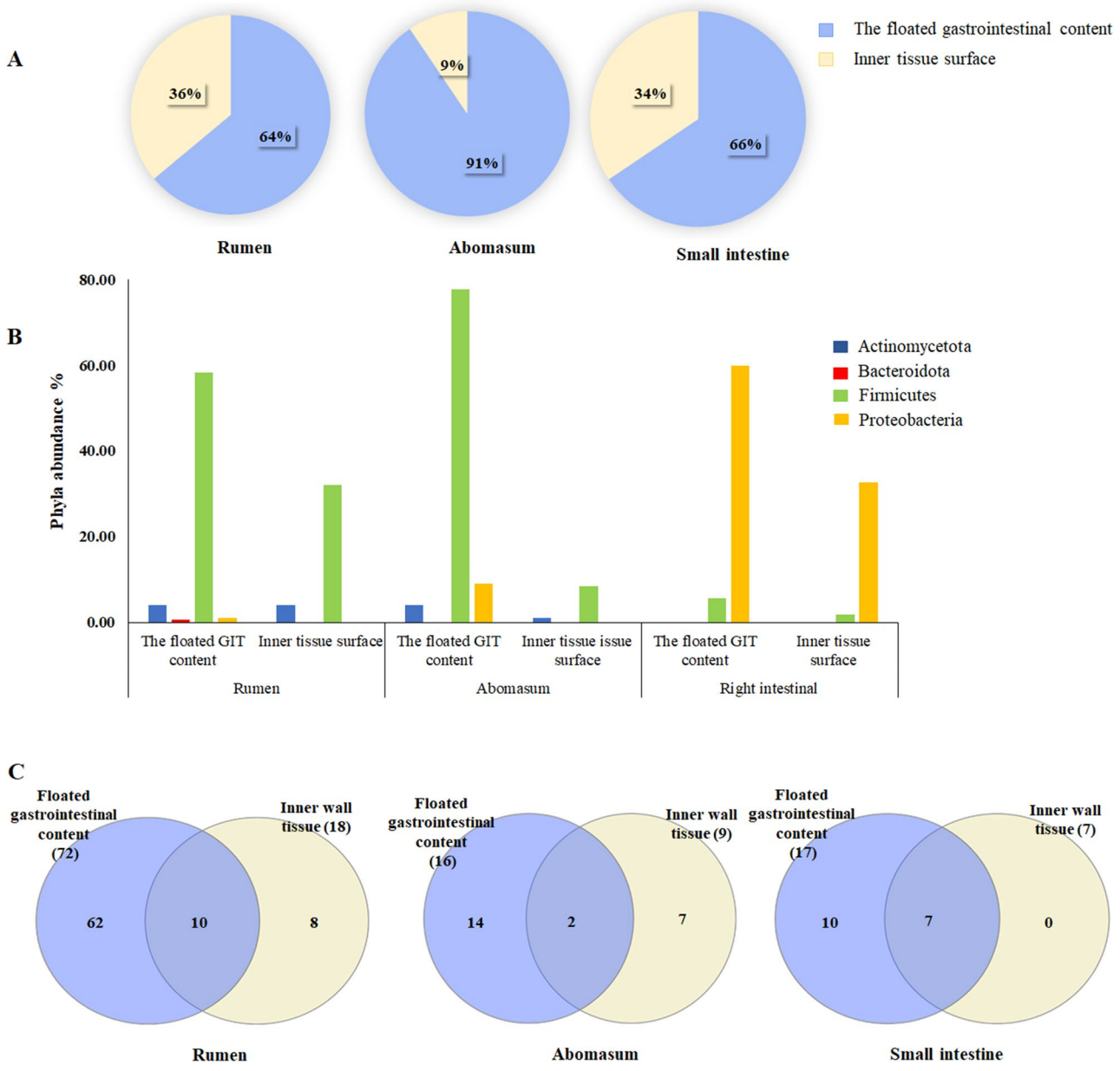

Fig. 3 Comparison of culturable bacteria attached to the inner wall and the luminal content across the rumen, abomasum, and small intestine of the camel. **A**: Percentage of total phyla in the inner wall and luminal content. **B**: Distribution of each phylum within each gastrointestinal section and compartment (inner wall vs. luminal content). Percentages were calculated independently for each section. **C**: Number of bacterial species shared between the inner wall and luminal content across the GIT compartments

sections. For example, *S. flexneri* and *E. fergusonii* were predominantly detected in the small intestine, highlighting niche specialization within the gut microbiome.

Culturable bacterial taxa shared across GIT sections

The culturable bacterial diversity was highest in the rumen, followed by the abomasum and small intestine. Three families—*Lactobacillaceae*, *Enterococcaceae*, and *Enterobacteriaceae*—were common to all three compartments (Fig. 5). Additionally, *Micrococcaceae*, *Streptococcaceae*, *Leuconostocaceae*, and *Bacillaceae* were shared between the rumen and abomasum.

At the genus level, *Enterococcus*, *Shigella*, and *Lactobacillus* were ubiquitous across all GIT sections. Five genera—*Streptococcus*, *Pseudomonas*, *Arthrobacter*, *Bacillus*, and *Pediococcus*—were shared between the rumen and abomasum, whereas no genera were found to be exclusively shared between the abomasum and small intestine. This pattern further underscores the strong compartmentalization and spatial structuring of microbial communities along the camel gastrointestinal tract.

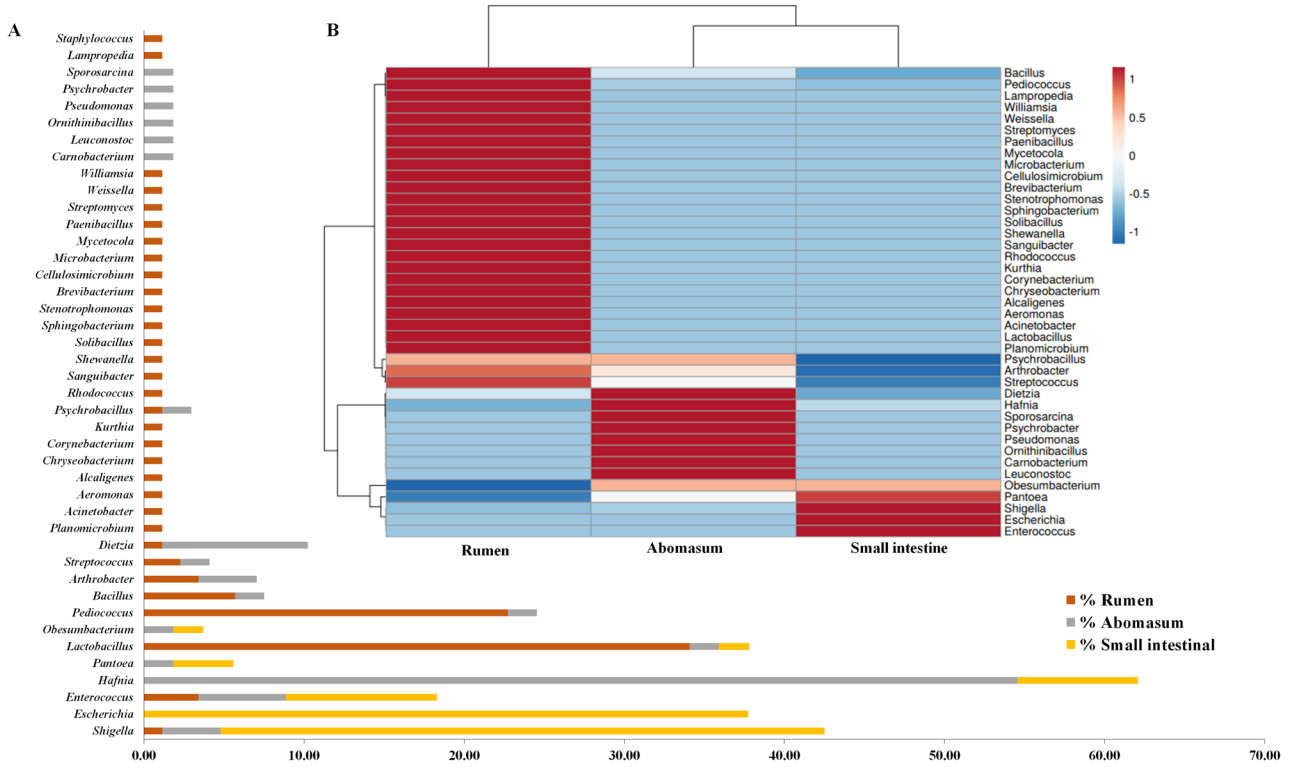

Fig. 4 Abundance of genera isolated in the culturable collection via culturomics from camel GIT compartments. **A**: Percentage of total genera and of each individual genus in the rumen, abomasum, and small intestine. **B**: Heatmap showing genus-level relative abundances of microbiota (columns) across rumen, abomasum, and small intestine segments (rows). Data were log-transformed and standardized (Z-scores) across compartments, with the color scale (−1 to +1) indicating values below or above the genus mean

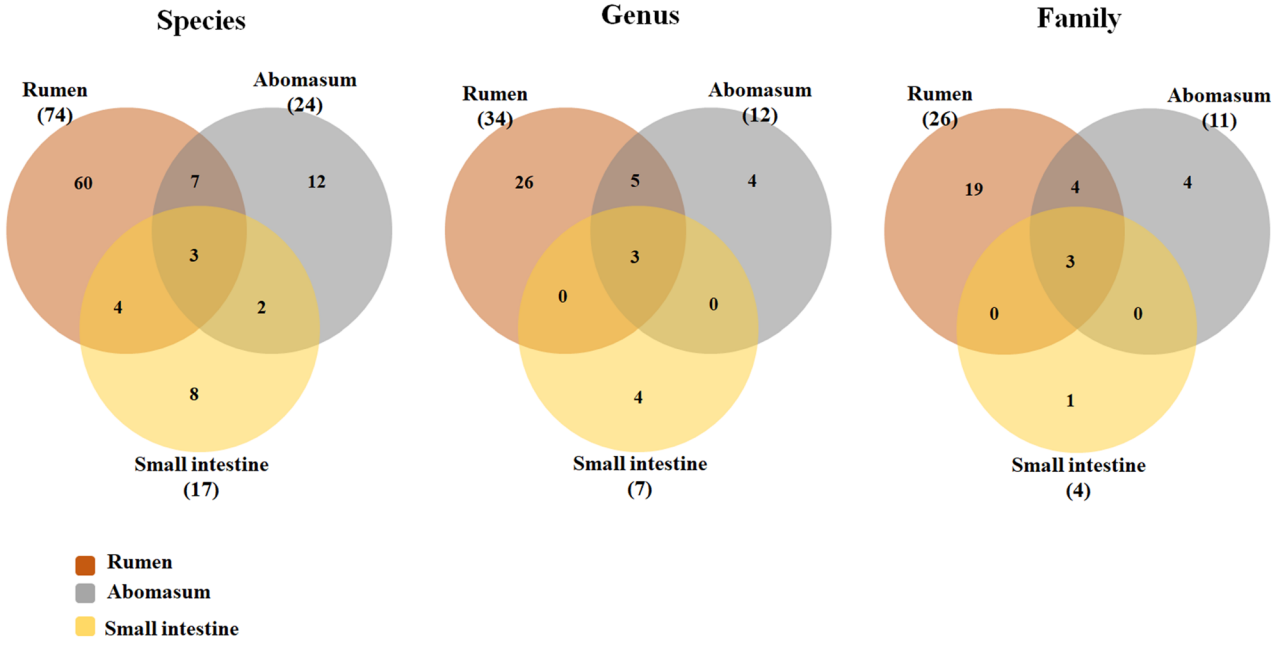

Fig. 5 Venn diagrams showing the overlap of bacterial taxa among the rumen, abomasum, and small intestine of the camel at three taxonomic levels: family, genus, and species. The numbers within each diagram represent the number of shared taxa identified at the corresponding taxonomic level

Species-level overlap across compartments was limited. Only three species—*Lactobacillus sakei*, *Enterococcus durans*, and *Enterococcus hirae*—were consistently recovered from all GIT sections. Seven species were shared between the rumen and abomasum, including *Lactobacillus curvatus*, *Pediococcus pentosaceus*, *Lactobacillus coryniformis*, *Bacillus altitudinis*, *Arthrobacter citreus*, *Bacillus licheniformis*, and *Streptococcus lutetiensis*. The abomasum and small intestine shared two species, *Enterococcus lactis* and *Shigella dysenteriae*, while four species—*Enterococcus faecium*, *Shigella flexneri*, *Shigella sonnei*, and *Enterococcus mundtii*—were common to the rumen and small intestine.

Moreover, PCoA analysis based on Bray-Curtis dissimilarity revealed distinct microbial groupings that separated the three GIT sections into separate clusters (Fig. 6A). PERMANOVA analysis confirmed that these differences were statistically significant ($p = 0.004$). Microbial diversity across the GIT showed a continuous pattern: the rumen exhibited the highest Shannon-effective diversity (e^H) at 38.84, whereas the abomasum and small intestine had much lower values of 8.72 and 6.79, respectively, indicating a steady decline in microbial diversity along the digestive tract (Fig. 6B). This trend was also reflected in effective richness, with the rumen presenting the maximum value of 0.84 compared to 0.69 in both the abomasum and small intestine. These findings suggest that the rumen serves as the primary reservoir for culturable microorganisms in the camel digestive system. Overall, alpha and beta diversity metrics demonstrate that microbial communities establish distinct patterns across the different gut sections.

Among the cultured isolates, four strains exhibited less than 98.65% 16S rRNA gene sequence identity with their closest type strains, suggesting that they may represent novel bacterial species. One isolate, affiliated with *Corynebacterium amycolatum*, showed 97.75% identity (GenBank accession no. MT013398), the others shared 96.11% similarity with *Lampropedia hyalina* (GenBank accession no. OR186636), *Microbacterium invictum* (GenBank accession no. PP029304) with 97.92% identity, and *Enterococcus hirae* (GenBank accession no. PP029288) with 95.80% identity. These isolates were obtained from the rumen under facultative anaerobic conditions at 37 °C and pH 6.0–6.4 using YCFA medium. On YCFA agar, the *Corynebacterium*-related strain produced small (1–2 mm), opaque, cream-colored, convex colonies with smooth margins, whereas the *Lampropedia*-related strain formed translucent, circular colonies with a slightly mucoid texture. The Microbacterium-related strain formed small to medium-sized (1–3 mm), opaque, yellowish to cream-colored, circular colonies with smooth edges and a convex elevation, whereas the Enterococcus-related strain produced small (≈1 mm), opaque, grayish-white, circular colonies with smooth margins and a slightly raised profile. Whole-genome sequencing and comparative genomic analyses (e.g., ANI and DDH) are planned to confirm their taxonomic status as potentially novel species.

Media-specific isolation of bacterial diversity

To recover a broad range of facultative and aerobic culturable taxa, six culture media were employed: YCFA, YCFA+S, YCFA+2G, YCFA+2GS (targeting fastidious

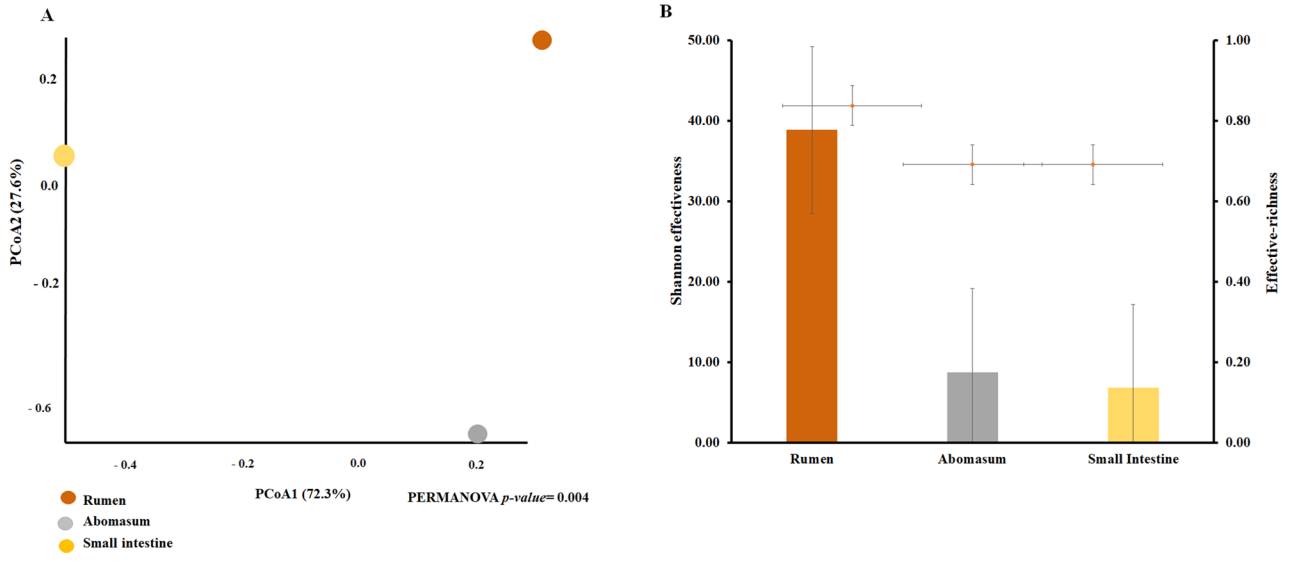

Fig. 6 Bacterial diversity estimated from the total culture collection obtained via culturomics across camel gastrointestinal tract (GIT) compartments. **A**: PCoA based on Bray-Curtis dissimilarity showing microbial community composition across the rumen, abomasum, and small intestine. **B**: Effective microbial richness and Shannon effective number of species in the three GIT sections

bacteria), and two general-purpose media nutrient agar (NA) and tryptic soy agar (TSA). YCFA yielded the highest number of isolates ($n = 701$), followed by YCFA+2 G ($n = 117$), YCFA+2GS ($n = 90$), TSA ($n = 86$), NA ($n = 56$), and YCFA+S ($n = 55$).

The YCFA medium supported the isolation of diverse taxa including approximately 31% of total *Firmicutes*, 95% of *Proteobacteria*, 38% of *Actinomycetota*, and all cultured *Bacteroidota* strains (Fig. 7A; Supplementary Table S6). Notably, YCFA enabled recovery of a broad phylogenetic range, including *Arthrobacter gandavensis*, *Bacillus haynesii*, *Cellulosimicrobium cellulans*, *Chryseobacterium aquaticum*, *Corynebacterium amycolatum*, *Escherichia marmotae*, *Kurthia gibsonii*, *Lampropedia hyaline*, *Obesumbacterium proteus*, and *Psychrobacter maritimus* among others.

Differential media enabled isolation of specific taxa. For example, YCFA+S enriched for *Bacillus simplex*, *Dietzia maris*, *Sporosarcina psychrophila*, *Streptococcus caballi*, and *Williamsia limnetica*. YCFA+2 G favored *Enterococcus gilvus* and *Mycetocola zhadangensis*, while YCFA+2GS enabled isolation of *Enterococcus avium* and *Streptococcus equinus*.

Hierarchical clustering based on taxonomic recovery patterns (Fig. 7B) revealed two major clusters: one composed exclusively of YCFA-related isolates, and another subdivided into five subclusters including general-purpose and modified YCFA media. The YCFA medium formed a distinct cluster with the highest taxonomic richness (Figs. 7B, and 8A), underscoring its utility for recovering diverse facultative anaerobes from the camel GIT.

The findings showed that PCoA analysis based on Bray–Curtis dissimilarity revealed distinct medium-specific patterns, with each culture medium forming a separate cluster (Fig. 8A). PERMANOVA analysis confirmed that these differences were statistically significant ($p = 0.001$).

Finally, diversity indices revealed pronounced medium-specific differences. YCFA exhibited the highest Shannon-effectiveness (20.94), followed by YCFA+S (9.87), whereas YCFA+2 G and YCFA+2GS showed substantially lower values (3.30 and 3.85, respectively) (Fig. 8B). Effective richness was greatest in NA (0.80) and YCFA (0.77) but declined sharply in YCFA+2 G (0.43) and YCFA+2GS (0.53). Collectively, these results demonstrate that culture medium composition profoundly influences the

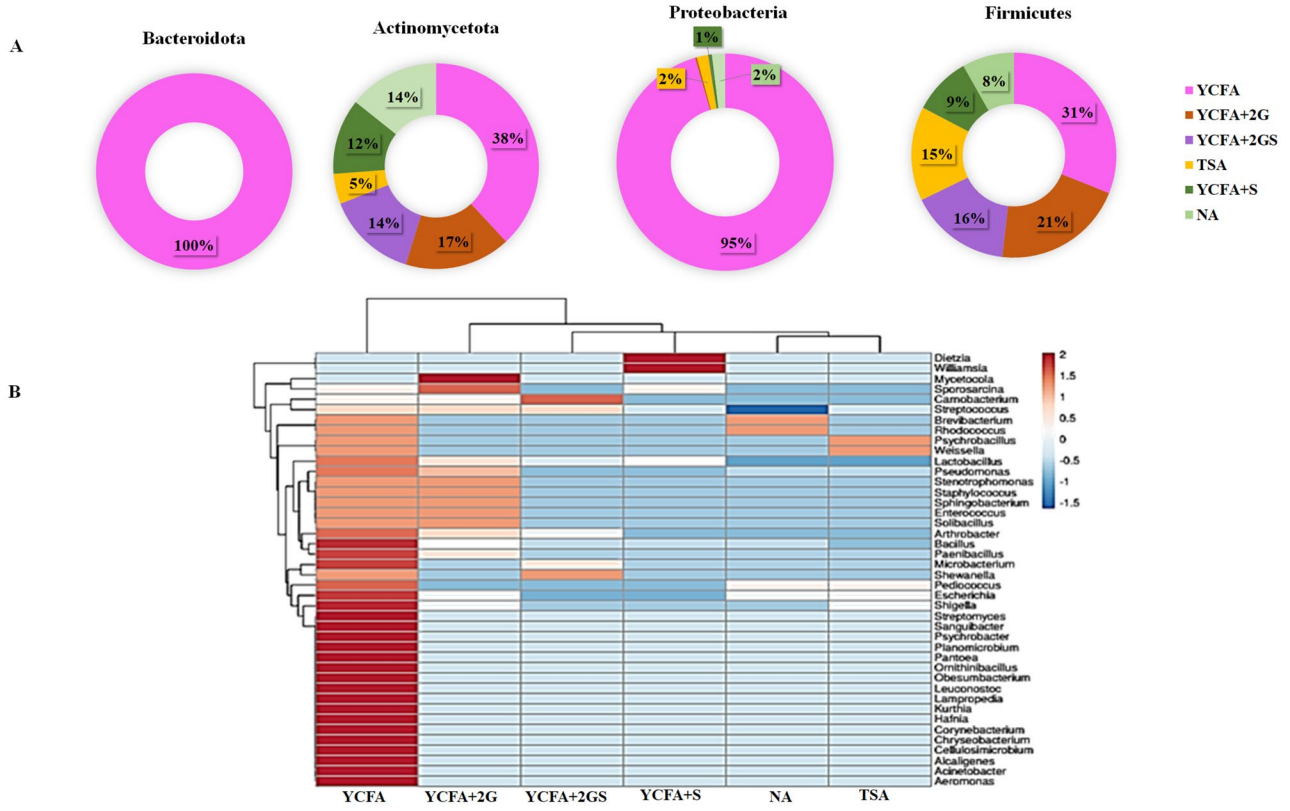

Fig. 7 **A**: Abundance and comparison of phyla retrieved from the total culture collection via culturomics in different specific (YCFA, YCFA+S, YCFA+2G, and YCFA+2GS) and general (NA and TSA) media under laboratory conditions. **B**: Heatmap diagram representing the abundance of microbiota composition at the genera (%) level (rows) across various specific and general media (columns). Data were log-transformed and standardized (Z-scores) across compartments, with the color scale (−1 to +1) representing values below or above the genus mean.

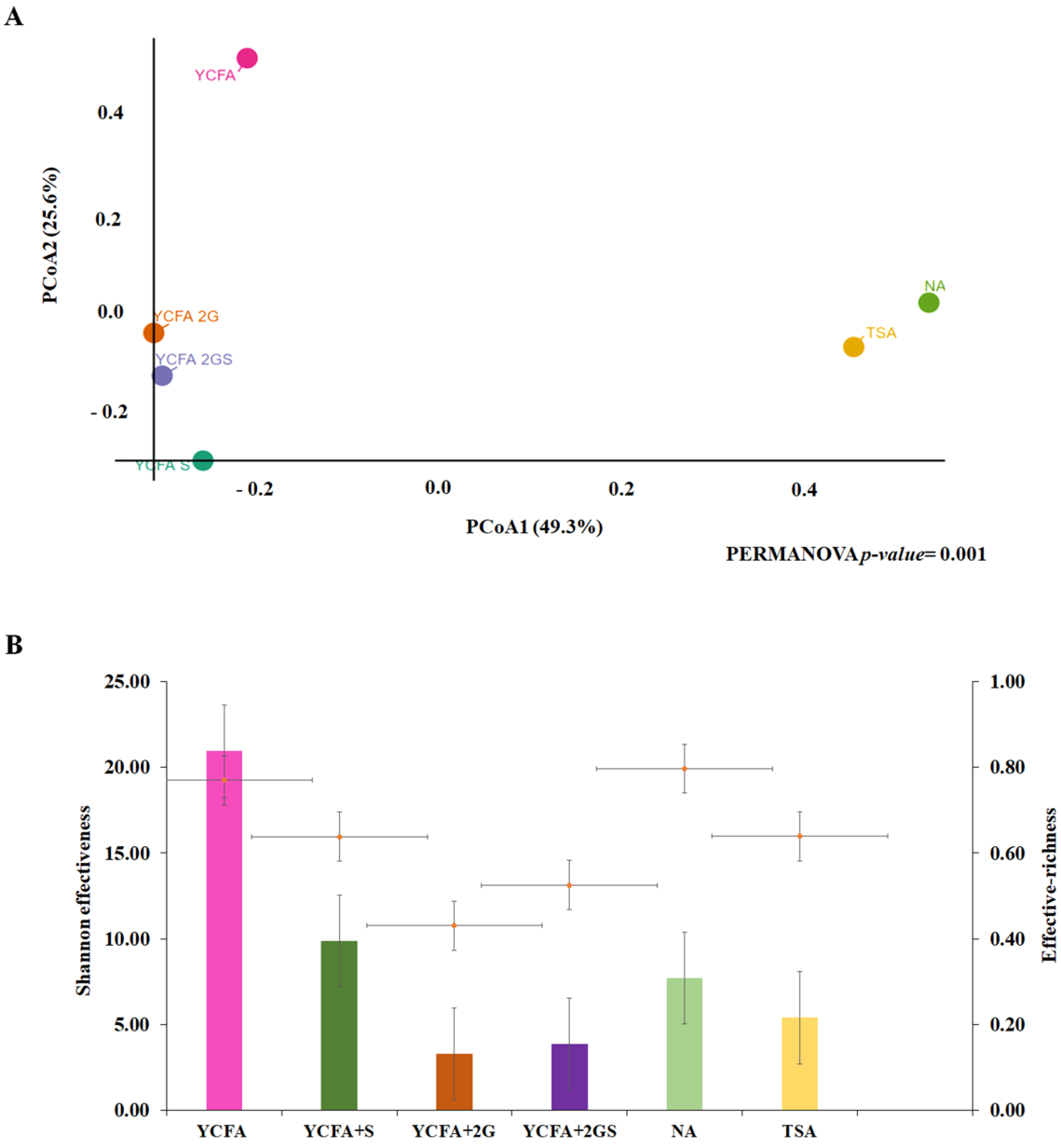

Fig. 8 Bacterial diversity estimated by 16S rRNA gene amplicon sequencing from the total culture collection obtained via culturomics using specific and general media. **A**: PCoA based on Bray–Curtis dissimilarity showing microbial community composition across YCFA-derived specific media (YCFA, YCFA+S, YCFA+2 G, and YCFA+2GS) and general media (NA and TSA). **B**: Effective microbial richness and Shannon effective number of species across the same media, highlighting differences in microbial diversity associated with culture conditions

culturable microbial community structure, with YCFA providing the most favorable conditions, while glucose supplementation markedly reduced both diversity and evenness.

Complementarity with metagenomics and Reference databases

Our findings underscore the complementary value of culturomics relative to metagenomic approaches. Whereas metagenomics excels at detecting DNA from both viable and non-cultivable microorganisms—including obligate anaerobes—culturomics uniquely enables the recovery of live bacterial isolates for subsequent functional characterization and biotechnological exploration [6, 16]. The limited overlap between culturomics and metagenomic data obtained from the camel GIT highlights the inherent methodological biases of each approach and reinforces the importance of integrating them to achieve a comprehensive understanding of gut microbial ecosystems [17].

Consistent with this notion, no overlap was observed between the genera recovered in this study and those identified in a previous metagenomic survey by

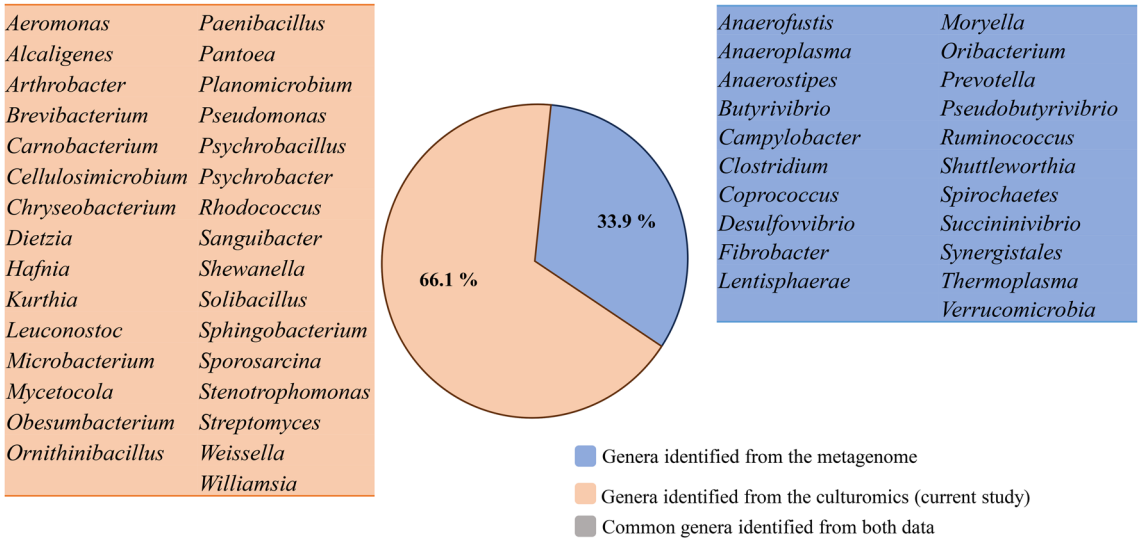

Fig. 9 Comparison between the genera identified in the current culturomics study and the metagenomic data from the camel gastrointestinal tract [8]

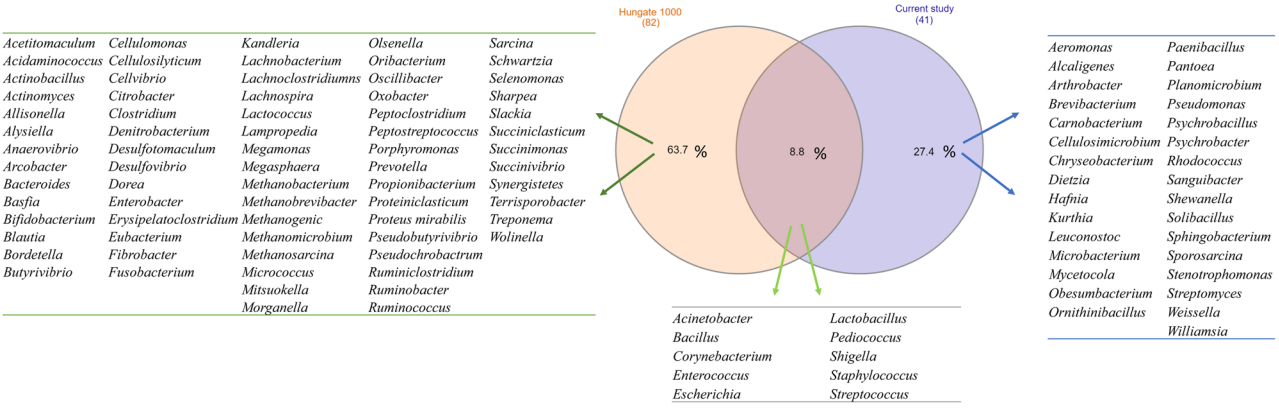

Fig. 10 Comparison between the genera identified in the current culturomics study and those from the hungate 1000 project 18 on the camel gastrointestinal tract

Gharechaee et al. [8], which predominantly detected strictly anaerobic taxa. In contrast, our culturomic strategy recovered 42 genera under aerobic or facultative anaerobic conditions, whereas the metagenomic study reported 21 mostly anaerobic genera, further emphasizing the complementary yet selective nature of both methods and the necessity for integrative multi-omics approaches (Fig. 9).

A further comparison with the Hungate1000 reference database [18], which catalogs gastrointestinal microbes from diverse herbivores hosts, revealed only limited overlap. Of the 82 genera listed in Hungate1000, merely 8.8% were shared with our dataset. In contrast, 27.4% of the genera isolated via culturomics in this study were absent from Hungate1000, while 63.7% of the Hungate1000 genera were not detected in our samples (Fig. 10). This pronounced discrepancy likely reflects the anaerobic cultivation bias of the Hungate1000 project and further underscores both the distinctiveness of the camel gut microbiome and the added value of culturomics in capturing less-characterized or previously unrecognized taxa. Collectively, the comparison between culturomic data, metagenomic profiles, and the Hungate1000 collection highlights culturomics as a powerful complement to genome-based approaches, enabling the recovery of live, functionally relevant species that are often overlooked in sequencing-only surveys.

Discussion

This study provides novel insights into the culturable bacterial diversity of the camel GIT, focusing on aerobic and facultative anaerobic bacteria across the rumen, abomasum, and small intestine. Using a high-throughput culturomics approach with tailored culture media, we successfully isolated 97 bacterial species spanning 42 genera and four phyla, including two putatively novel taxa. These findings highlight the camel's distinctive microbial ecology, its physiological adaptation to arid

environments, and the value of culturomics as a powerful complement to metagenomics for exploring host-associated microbial communities [1, 2, 16].

Shannon and Simpson diversity indices revealed marked heterogeneity among GIT compartments, with the rumen exhibiting the highest bacterial diversity and the small intestine the lowest. Notably, members of *Bacteroidetes*, typically abundant in other ruminants [19–21], were markedly underrepresented in camels. This observation aligns with findings in high-producing dairy cattle and comparative studies of lean versus obese humans, where *Bacteroidetes* abundance was substantially lower relative to other phyla [22–24]. The Firmicutes-to-Bacteroidetes ratio in camels thus appears to deviate from the general pattern described in other ruminants, suggesting host-specific microbial adaptations. Such adaptations likely reflect evolutionary pressures imposed by arid environments and underscore the importance of ecological and physiological context when interpreting gut microbiome structures.

The low diversity observed in the small intestine likely results from acidic pH, bile and pancreatic secretions, and rapid digesta transit, which collectively limit bacterial colonization [16, 25, 26]. These compartment-specific patterns reflect distinct ecological niches within the camel GIT and are consistent with findings in other ruminants, including cattle, sheep, and goats [19, 27].

Members of the *Bacteroidetes* phylum encode a broad repertoire of debranching and oligosaccharide-degrading enzymes, whereas *Firmicutes* are enriched in cellulases and hemicellulases. Together, these lineages appear to play complementary roles in lignocellulose degradation, ensuring efficient fiber breakdown and nutrient utilization in the camel GIT—particularly under arid environmental conditions. Certain *Proteobacteria* genera contributed to nitrogen metabolism and gut priming for strict anaerobes, while others were closely related to opportunistic or pathogenic species [27–29]. Overall, the microbial composition mirrors trends in other ruminants, where *Firmicutes* and *Proteobacteria* dominate nutrient metabolism and maintain gut homeostasis. Furthermore, compositional differences between mucosal and luminal microbiota highlight the significance of microhabitat-specific adaptation within the gut ecosystem [30].

The culturomics approach also facilitated the isolation of stress-tolerant and facultatively anaerobic species, including *Bacillus* spp., *Lactobacillus* spp., *Lactobacillus sakei*, and *Pediococcus pentosaceus*. These taxa likely exhibit cellulolytic, bile-tolerant, and acid-resistant traits, enabling them to survive fluctuations in oxygen levels, osmotic pressure, and nutrient availability typical of desert habitats. Such physiological versatility not only supports microbial persistence and functionality in the harsh camel GIT environment but also underscores their potential for fiber degradation, pathogen inhibition, and biotechnological applications. Of particular note was the identification of several genera—including *Sporosarcina*, *Carnobacterium*, *Psychrobacter*, and *Pseudomonas*—which thrived predominantly in the acidic environment of the stomach. This suggests a metabolically versatile microbial repertoire that may contribute to camels' adaptation to harsh desert conditions. These taxa are associated with functional traits such as fiber degradation, pathogen inhibition, phytate hydrolysis, hydrocarbon bioremediation, and lactic acid production, emphasizing their ecological and biotechnological relevance [31–33].

Among the media tested, YCFA-based medium proved particularly effective, enabling the recovery of 88.64% of all identified species. This demonstrates its high efficiency in supporting the growth of diverse and often fastidious bacterial taxa, including rare representatives that may be underrepresented in culture-independent surveys [1].

Our findings suggest that targeted enrichment of beneficial microbes in the rumen and abomasum could enhance fiber digestion and support host resilience under water- and nutrient-limited conditions, reflecting finely tuned microbial adaptation to extreme environmental pressures. The isolation of rare or novel taxa further opens avenues for discovering new enzymes, antimicrobial compounds, or probiotic candidates relevant to agriculture and biotechnology. Although this study did not assess sex-related microbiome differences, future investigations should address this aspect, as host sex is known to influence gut microbiota composition in other ruminants. Overall, our results demonstrate that culturomics greatly expands our understanding of the camel gut microbiota, identifies novel taxa, and provides valuable functional insights into microbial adaptation to extreme arid environments.

Limitations and future directions

Despite its strengths, this study has several limitations. First, by focusing on aerobic and facultative anaerobic bacteria, many obligate anaerobes—dominant inhabitants of the camel rumen and abomasum—were likely excluded [22]. Expanding culturomics protocols to encompass strict anaerobic cultivation strategies is essential to capture the full extent of microbial diversity. Second, the limited sample size-three camels from a single geographic region—restricts broader generalizations. Future studies involving larger cohorts from diverse ecological, environmental, and dietary contexts are necessary to unravel the ecological drivers of microbial diversity in camels [34, 35]. While taxonomic identification provides a valuable foundation, functional validation of the isolated strains remains a critical next step. Assessing their

enzymatic activities, resistance mechanisms, and probiotic properties will be essential for translating these findings into practical applications in animal health, feed efficiency, and biotechnology [36–38]. Integration with multi-omics platforms—such as metatranscriptomics, metabolomics, and proteomics—will further elucidate microbial functions and host–microbiome interactions [1, 39, 40].

Conclusion and broader implications

Q2

This study highlights the camel gastrointestinal tract as a rich yet underexplored reservoir of microbial diversity, offering new perspectives on microbiome evolution, environmental adaptation, and potential biotechnological utility. The recovery of previously uncharacterized and potentially novel taxa underscores the scientific and industrial relevance of culturomics, particularly in non-model hosts and extreme environments [41, 42]. As global interest in microbial innovations for sustainable agriculture, animal health, and biotechnology grows, characterizing these unique microbial communities could provide novel tools to enhance resilience in arid and resource-limited systems. Integrating culturomics with anaerobic and multi-omics methodologies will enable a more holistic and function-oriented understanding of host-associated microbiomes [43]. Such interdisciplinary approaches are pivotal for unlocking the full ecological and application potential of microbial ecosystems—ultimately advancing sustainable development, animal productivity, and microbial biotechnology [15].

Supplementary Information

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Supplementary material 1

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Author contributions

Author Contributions Raha Abedini: Contributed to the acquisition, analysis, and interpretation of data; drafted the manuscript and provided substantial revisions. Ghasem Hosseini Salekdeh: Contributed to the conception and design of the study; reviewed and revised the initial draft of the manuscript. Maryam Hashemi: Contributed to the conception and design of the study; participated in data interpretation; reviewed and revised the initial draft critically for important intellectual content.

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Data availability

The 16S rRNA gene sequences obtained from all bacterial isolates retrieved in this study have been deposited in NCBI GenBank as Dataset 1. Corresponding accession numbers are provided in Supplementary Table S2.

Declarations

Competing interests

The authors declare no competing interests.

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