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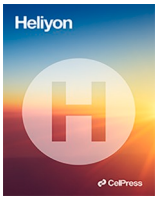

Research article

Profiling the dynamic adaptations of CAZyme-Producing microorganisms in the gastrointestinal tract of South African goats

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ABSTRACT

The gastrointestinal tract of goats serves as a habitat for anaerobic microbial populations that work together to break down complex plant material, including lignocellulose. This study explored the microbial diversity and metabolic profiles across different gastrointestinal tract compartments. Significant diversity differences among the compartments were observed (ANOSIM $p < 0.006$), with the abomasum showing a distinct species composition and a decreased alpha diversity (Mann-Whitney/Kruskal-Wallis test $p = 0.00096$), possibly due to its acidic environment. Dominant microbial phyla included Proteobacteria, Bacteroidetes, and Firmicutes, with Proteobacteria being the most prevalent in the abomasum (50.06 %). Genera like *Proteus* and *Bacteroides* were particularly prominent in the rumen and reticulum, highlighting their significant role in feed degradation and fermentation processes. Over 65 % of genes at Kyoto Encyclopedia of Genes and Genomes level 1 were involved in metabolism with significant xenobiotic biodegradation in the abomasum. The dbCAN2 search identified Glycoside Hydrolases as the most prevalent CAZyme class (79 %), followed by Glycosyltransferases, Polysaccharide Lyases, and Carbohydrate Esterases, with Carbohydrate-Binding Modules and Auxiliary Activities accounting for 1 % of the hits. Higher CAZyme abundance was observed in the reticulum and omasum compartments, possibly due to MAGs diversity. In conclusion, the gastrointestinal tract of South African goats harbors diverse CAZyme classes, with Glycoside Hydrolases predominating. Interestingly, higher CAZyme abundance in specific compartments suggested compartmentalized microbial activity, reflecting adaptation to dietary substrates.

1. Introduction

Ruminants, including goats (*Capra aegagrus hircus*), possess a complex gastrointestinal tract (GIT) comprising the rumen, reticulum, omasum, and abomasum, which house a diverse and dynamic microbial ecosystem [1–3]. This intricate mutual beneficial relationship between the host and the ruminal microbiome is crucial for the efficient fermentation of complex plant material ruminants consume, including lignocellulose [4,5]. Central to this process is the secretion of specialized enzymes known as Carbohydrate-Active enzymes (CAZymes) by the ruminal microbiome, facilitating the breakdown of complex plant cell walls into digestible nutrients [6–8]. While the importance of CAZymes in lignocellulosic biomass degradation is well-established, there remains a gap in understanding the

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specific genomic makeup and functional potential of the microbial communities within the goat GIT, particularly in the context of South African goats.

This knowledge gap presents an opportunity to elucidate the genetic basis of CAZyme production and its implications for lignocellulosic biomass utilization in this unique population [9–12]. This knowledge gap presents an opportunity to elucidate the genetic basis of CAZyme production and its impact on lignocellulosic biomass utilization in this unique population [13–16]. One key challenge in harnessing the potential of lignocellulosic biomass lies in its recalcitrant nature, characterized by cellulose's complex structure, lignin's presence, and hemicellulose composition [9,17,18]. Overcoming these challenges requires a deeper understanding of the microbial enzymes involved in biomass degradation and the genetic mechanisms underlying their production [19,20].

To address these gaps in knowledge, our study aims to characterize the metagenomic profiles of microbial communities within each compartment of the South African goats' GIT, focusing on the genomic abundance of CAZymes and their potential for lignocellulosic biomass degradation; investigate the functional roles of specific microbial taxa and their associated CAZymes in biomass conversion, with a particular emphasis on overcoming the recalcitrant nature of lignocellulosic substrates; and then assess the impact of geographic location on the composition and functional potential of the goat GIT microbiome, hypothesizing that this factor influence CAZyme gene expression and microbial community dynamics. By rigorously testing these hypotheses, we aim to advance our understanding of the genetic basis of lignocellulosic biomass degradation in South African goats and pave the way for developing sustainable biotechnological solutions for biomass conversion and potential bioenergy production.

2. Methods and materials

Ethical statement

All the necessary research permits and relevant ethical committee approval were given. Ethical guidelines from College of Agricultural and Environmental Sciences Animal Research Ethics Committee (CAESAREC) of the University of South Africa approved this study and allocated reference number: 2021/CAES_AREC/097 under Dr. A Wilson. The Unisa SOE Ethics Review committee also approved the study and allocated the reference number 2020/CSET/SOE/028 under Prof. E Onyari-Benecha. Ensuring humane treatment of the animals during slaughtering and compliance with regulations The Chief State Veterinarian of South African Agriculture and Rural Development, Dr S Kamudyariwa, also approved this study and allocated it the reference number UNISA-01-2021-KJR.

2.1. Sample information and collection

A total of 10 Saanen Boer domestic goats, aged between 2.5 and 3 years, were selected based on weight and dental development to ensure they were in a similar physiological stage. This age range was chosen under the hypothesis that older goats would harbor a microbiome more adapted to efficiently breaking down plant material. The goats were sourced from two provinces in South Africa, with nine goats procured from three free-range farmers. As a result, the goats were kept under free-range conditions, allowing for natural grazing behavior and diet selection. Specifically, three goats were sourced from Springfontein ($30^{o}15'14''$S $25^{o}42'14''$E), Free State Province, Verkeerdevlei ($28^{o}50'$S $26^{o}47'$E), Free State Province, and Moremela ($24.6663^{o}50'$S 30.801°E), Mpumalanga Provinc e (Supplementary table S1. An additional goat, G10 from Volkrust ($27^{o}22'$S $29^{o}53'$E), Mpumalanga province, was included as a control, with dietary conditions carefully controlled and nutritional information provided in supplementary table S2. According to our knowledge, no antibiotics or drugs were administered to the animals before procurement.

The goats were taken to a registered abattoir and slaughtered following the guidelines set forth by the National Council of Societies for the Prevention of Cruelty to Animals (NSPCA) by a designated butcher. Each compartment (rumen, reticulum, omasum, and abomasum) from the stomach was sampled aseptically using a spatula and labelled accordingly. The samples were transported to the University of South Africa laboratory on ice and stored at 4 °C for a duration not exceeding 24 h following metagenomic DNA extraction.

2.2. Metagenomic DNA extraction and sequencing

A total of 40 samples (4 compartments per goat) were weighed between 146 and 150 mg and were extracted using the Quick-DNA fecal/soil microbe miniprep kit (Zymo Research, Irvine, CA) following the manufacturer's instructions. The quantity of extracted DNA was evaluated using the Qubit 3.0 Fluorometer (Invitrogen, Carlsbad, CA, USA). The metagenome library for sequencing was conducted using the MGIEasy Universal DNA Library Set (MGI Tech Co., Ltd, Shenzhen, China) according to the manufacturer's instruction and then sequenced using the MGI DNBSEQ-400 sequencer with 2x150bp reads.

2.3. Diversity and taxonomy of the microbial community in various compartments

Raw metagenomic sets of paired-end reads were imported to the Nephele platform (version 2.32.1) [21] for analysis. Additional trim and filters were run on default settings, and the system outputs were specified for TEDread fastq files, gene-based taxonomic profiling, and taxonomic annotations of scaffolds. At the same time, the Kyoto Encyclopedia of Genes and Genomes (KEGG) database was used for metabolic pathways [21]. The merged taxa tables were then uploaded onto the MicrobiomeAnalyst database. The total read counts amounted to 316 315 689 with an average of 790 7892 per sample and maximum counts of 15069618 with a minimum of

48 739 77 per sample. To filter the data, the low count filter minimum was set at four, and the prevalence in samples was 20 % with mean abundance value, while the low variance removal filter was 10 % based on the inter-quantile range (IQR). A total of 2139 low abundance features were removed based on prevalence, and approximately 668 low variance features were removed based on IQR—the number of features that remained after the data filtering step was 6003. The data was further normalized using the cumulative sum scaling (CSS) option, and the actual abundance stacked bar was generated for taxonomic profiling of compartments at the phylum and genus levels, merging small taxa with count <10 based on total read (n = 10). Furthermore, alpha and diversity plots were generated [22].

2.4. Functional profiles of whole metagenomes of each compartment group

Raw metagenomics reads were imported to Nephele (version 2.32.1) to analyze the diversity and relationship of the functional annotations [21] using KEGG, and the resulting gene abundance table was then imported to Statistical Analysis of Metagenomic Profiles (STAMP) [23,24] in order to determine the abundance and significant gene families in each compartment.

2.5. Classification of Metagenome-Assembled Genomes and CAZymes identification

To facilitate this classification, the utilization of the DOE Systems Biology Knowledgebase (KBase), an open-source software and data platform that promotes data sharing, integration, and analysis of microbes, plants, and their communities, was employed [25]. The Quality Control on the sequence reads was performed on all 40 metagenomic read libraries using FastQC v.011.9, and the resulting reads were then trimmed using the Trimmomatic v0.36. This was further run on the FastQC to assess the quality of trimmed reads. The trimmed sequences were assembled into contiguous fragments (contigs) to create the scaffolding of whole genomes using metaSPades v3.13, MegaHit v.1.29, and IDBA-UD v1.1.3 [26]. Assemblies from all three methods were merged using KB v1.0.1 and compared for assembled contig distribution and quality using the Quality Assessment Tool (QUAST) report. The assembly platform that resulted in the highest quality of contigs was chosen for each library. The assembled contigs were clustered into bins, each corresponding to a putative population genome using MetaBAT2 v1.7, CONCOCT v1.1, and MaxBin2 v2.2.4. DAS Tool improved the binning quality using consensus assignments from the binned contigs output by MetaBAT2, CONCOCT, and MaxBin2. We explored the abundance and diversity of CAZymes within the Metagenome-Assembled Genomes (MAGs) from the various compartments of the goat's GIT, focusing on their potential role in lignocellulosic biomass degradation. The assembled contigs clustered into bins, each corresponding to a putative population genome, resulted in 180 Metagenomic Assembled Genomes (MAGs). CheckM v1.0.18 analysis revealed high completeness (averaging 98.40 %) and low contamination (averaging 0.57 %) of MAGs. A 95 % or higher completeness score and contamination less than 5 % was generally considered good [27,28]. Hence, this confirmed that the assembly process was successful and that the assembled genomes were relatively pure and did not contain significant genetic material from other organisms or sources [27]. The quality and completeness of all the MAGs are represented in Table S2. The MAGs filtered by quality were extracted to individual genome assembly objects with BinUtil v1.0.2. To obtain KBase [25] genomes, the genes were annotated using RAST and classified using the Genome Taxonomy Database (GTDB-Tk). To construct a phylogenetic distribution analysis of the predicted CAZymes and draw a correlation between the GIT microbiome, the MAGs were taxonomically classified based on a concatenated protein-based phylogeny representing more than 250 000 prokaryote genomes using the GTDB-Tk. The phylogenetic tree was generated using iTOL [29,30]. We then examined all 180 MAGs for CAZyme diversity related to lignocellulosic biomass degradation, such as cellulase, lignin, hemicellulase, and pectinase/esterase according to the dbCAN2 database with an E-Value of 1e-15[26]. Parameters were set to profile all groups of CAZymes, including cellulosome Fams, Glycoside Hydrolases (GH), Glycosyl Transferases (GT), Carbohydrate-Binding Modules (CBM), Carbohydrate Esterases (CE), Polysaccharide Lyases (PL) and Auxiliary Activities (AA) with an E-Value of 1e-15 [26,31,32].

2.6. Statistical Analysis

Alpha diversity metrics, including Chao1 and Shannon Observed_species, were utilized to measure species richness and evenness across different compartments. Statistical significance was determined using a non-parametric test, specifically the Mann-Whitney *U* test for comparing two independent groups and the Kruskal-Wallis test for comparing three or more independent groups. The post-hoc test analysis was also conducted using the Tukey-Kramer test to identify specific group differences in cases where significant differences were observed. All these tests were done on the web-based MicrobiomeAnalyst using the Marker Data Profiling feature [22]. In the multivariate analysis, Principal Coordinate Analysis (PCoA) was conducted using the Bray-Curtis Index as a distance metric in MicrobiomeAnalyst. The number of dimensions (principal coordinates) used was determined based on Eigenvalues and scree plots, which indicate the variance each principal coordinate explains. To achieve PCoA in MicrobiomeAnalyst, the software utilizes internal algorithms and computations to perform the analysis. The significance of differences in microbial community composition among groups was assessed using the Analysis of Similarities (ANOSIM) statistical method with a conventional threshold of $p < 0.05$ considered significant [33,34]. Kruskal-Wallis H-test statistical test and the Turkey-Kramer Post-hoc test to the value of 0.95 was done on the KEGG functional annotation heatmap that was generated on STAMP [23,24]. The PCA was done using the SRplot [35] with an eclipse of 68 % confidence interval.

3. Results and discussion

3.1. The richness and diversity of the microbial community in various compartments

Comparative analysis of diversity among all compartments (beta-diversity), visualized by Principal Coordinate Analysis (PCoA) using the Bray–Curtis dissimilarity index, revealed clustering of microbial communities from adjacent GIT compartments, such as the reticulo-rumen, reticulum, and omasum, indicating related taxonomy composition (Fig. 1).

Conversely, OTUs from the abomasum were mainly separated, suggesting a distinct species composition. ANOSIM analysis confirmed significant compartment differences ($p < 0.006$; $R = 0.12261$). Consistent with prior research [33], the abomasum exhibited notably different microbial communities, suggesting physiological and environmental distinctions.

Alpha diversity analyses, including Chao1 (richness), Shannon index (diversity), and Observed_species (estimated OTUs), further elucidated microbial diversity profiles. The omasum displayed the highest Chao1 diversity (5756.4), followed by the reticulum (5599.6), rumen (5550.2), and abomasum (4698.3), indicating lower species richness in the latter (Fig. 2A).

The Shannon diversity (Fig. 2B) values varied slightly among compartments, with the rumen exhibiting the highest diversity (2.59), followed by the omasum (2.50), reticulum (2.49), and abomasum (2.29). Observed diversity (Fig. 2C) followed a similar trend, with the omasum having the highest values (5562.4), followed by reticulum (5401.6), rumen (5266.7), and abomasum (4161.6). Statistical analysis using Mann-Whitney/Kruskal-Wallis tests revealed significant differences in Chao1 diversity ($p = 0.00096$), indicating non-random variations among compartments.

However, Shannon diversity differences were not statistically significant ($p = 0.34399$), suggesting similar evenness across compartments. Meanwhile, like the Chao1 diversity, Observed diversity differences were statistically significant ($p = 0.00157$), indicating actual differences in species richness. These findings suggest decreased richness and evenness in the abomasum, attributed to its acidic environment resembling that of monogastric animals [33,36]. Acidic conditions have the potential to inhibit microbial growth and favor acid-tolerant species, as observed in prior goat studies [33].

3.2. Taxonomic classification of microbial communities in various compartments

The taxonomic analysis revealed that the compartments were dominated by Proteobacteria, Bacteroidetes, and Firmicutes phyla, as well as a notably higher abundance of unclassified phyla ($n = 10$). However, the distribution in all the compartments varied (Fig. 3A). The abomasum had the highest prevalence of Proteobacteria (50.06 %), followed by the rumen (30.52 %), reticulum (27.58 %) and the omasum (23.52 %). A notable decrease in Bacteroidetes was observed from the rumen to the abomasum. The rumen consisted of 23.58 %, the reticulum had 18.05 %, the omasum had 15.29 %, and the abomasum consisted of the least abundance at 13.9 %.

On the other hand, Firmicutes were relatively more abundant in the abomasum and least prevalent in the other compartments, particularly the reticulum and rumen. The Firmicutes accounted for 19.9 % of the abomasum, 18.55 % of the omasum, 17.49 % of the rumen, and 17.22 % of the reticulum. Actinobacteria was present in amounts of less than 1 % in the rumen, reticulum, and abomasum and approximately 1.39 % in the omasum. The remaining phyla were found in relatively lower levels in all compartments, with no

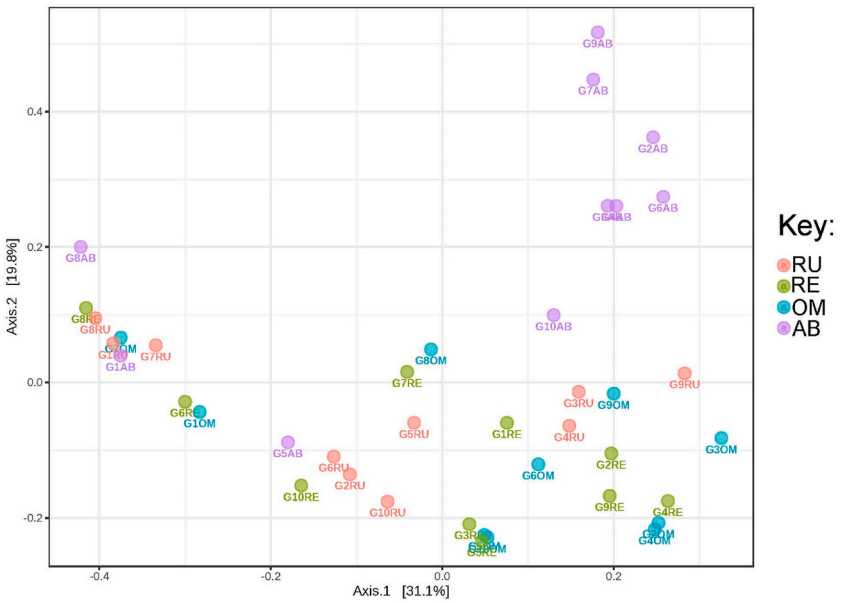

Fig. 1. Principal Coordinate Analysis (PCoA) plot with the compartment as the primary factor using the Bray-Curtis Dissimilarity index (ANOSIM $p < 0.006$).

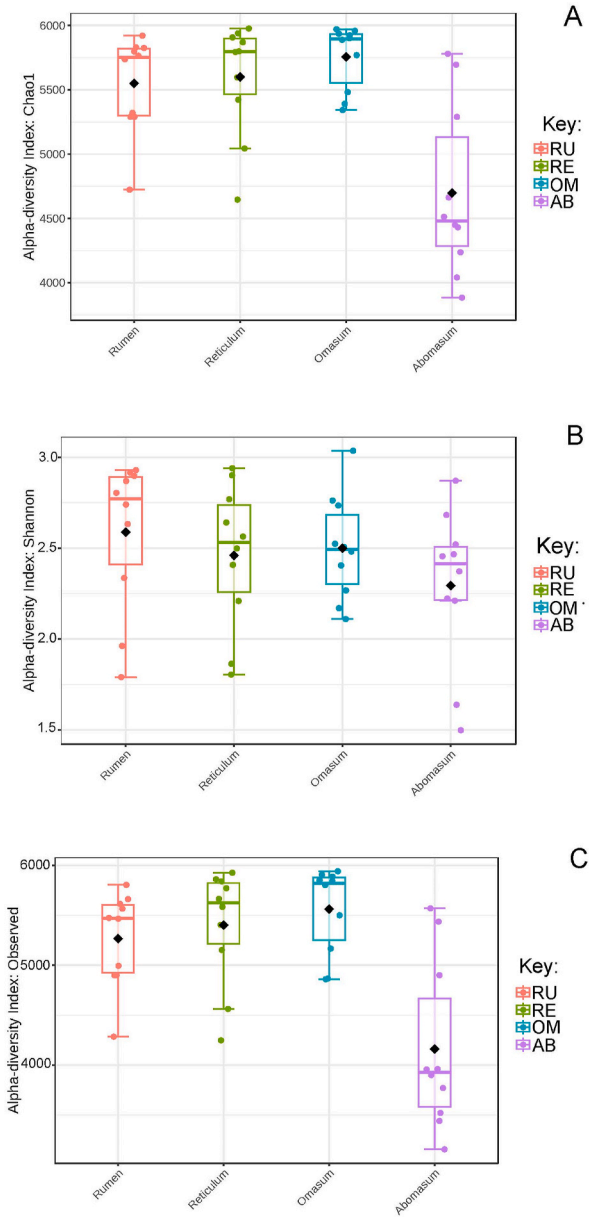

Fig. 2. **A)** Alpha diversity indices Chao1, **B)** Shannon, and **C)** Observed species derived from the rumen (RU), reticulum (RE), omasum (OM) and abomasum (AB).

phylum accounting for more than 0.6 % of the total biomass. The unclassified phyla were also present in relatively higher abundance in the omasum, reticulum, and rumen, accounting for 40.03 %, 35.2 %, and 26.77 % of total biomass, respectively, with the abomasum having the least abundance (14.71 %) among the other compartments. These findings suggest that the goats' digestive tract microbial communities are diverse and attributed to specific physiological aspects of each compartment. For example, a notable population of Proteobacteria has been reported to thrive in acidic environments, while Bacteroidetes thrive well in alkaline environments [36]. As the true stomach of ruminants, the abomasum is characterized by acidic and proteolytic conditions essential for the digestion of protein-rich feed [33,37]. The higher prevalence of Proteobacteria might be associated with their ability to thrive in low pH environments [36]. In other studies, Bacteroidetes, Firmicutes, and Proteobacteria were also found to be the primary dominant phyla [38–40]. In similar studies, Bacteroidetes was also found in higher abundance in the rumen compartment [33,40,41]. Anaerobic bacteria from the two major phyla, Bacteroidetes and Firmicutes, comprise most of the microbiome in a healthy GIT [42]. Bacteroidetes have a significant role in the breakdown of complex carbohydrates because of the number of genes that encode CAZymes [43]. Firmicutes are primarily responsible for degrading complex polymers, including cellulose, hemicellulose, and other fibrous materials [43]. Therefore, these two phyla and their functions in stimulating the breakdown of dietary fibers and other complex compounds are

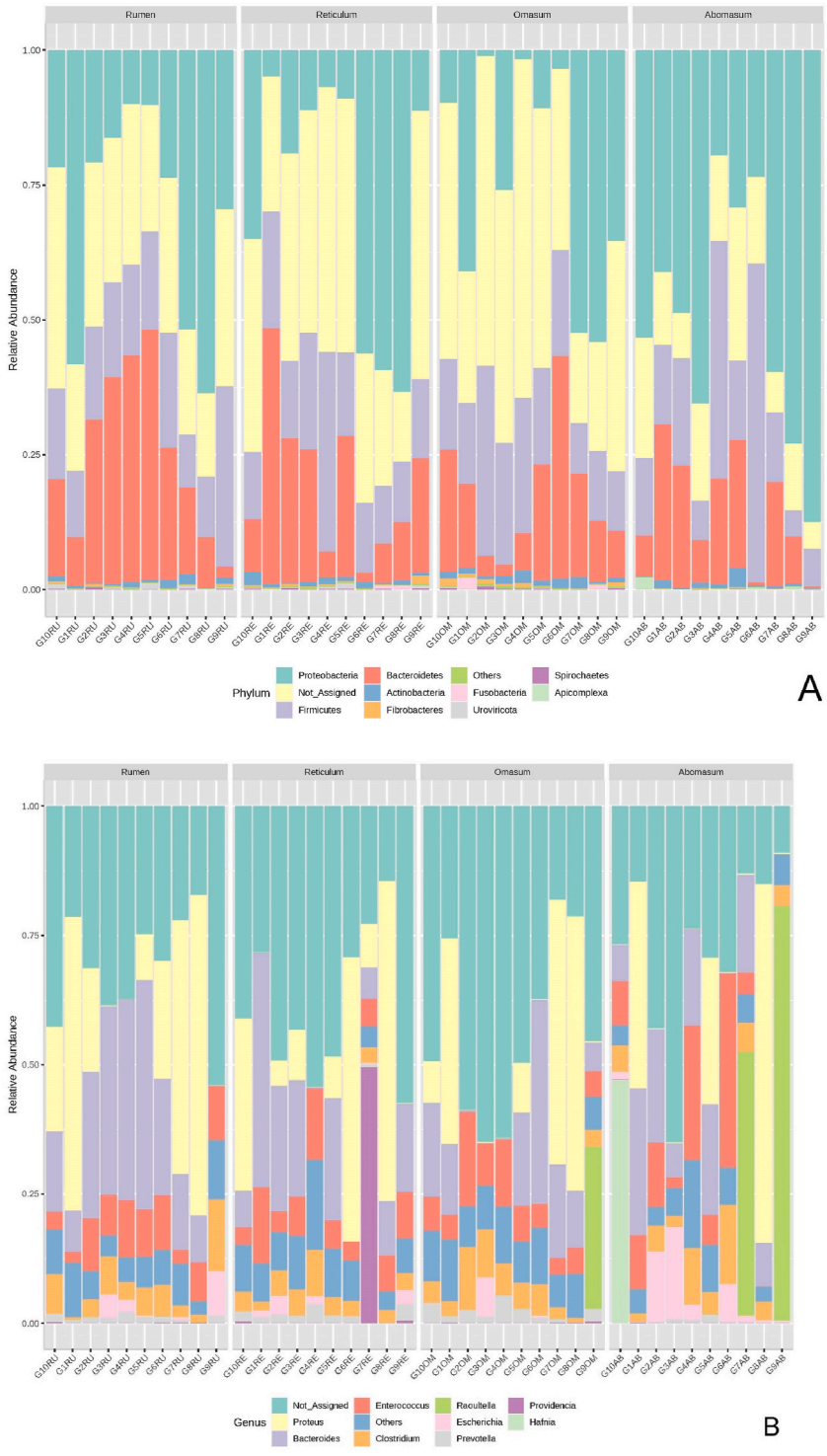

Fig. 3. A) The relative abundance of the ten most prevalent Phyla, **B)** Genus across all goat's compartments; Rumen, Reticulum, Omasum, Abomasum.

indicators of a healthy GIT.

The microbial composition analysis across different compartments of the goats' GIT also revealed variations in the abundance of genera (Fig. 3B). Among the analyzed genera, *Proteus*, *Bacteroides*, *Enterococcus,* and *Clostridium* were prominent across all compartments. Among the identified taxa, *Proteus* and *Bacteroides* genera were the most prevalent in the rumen, representing approximately

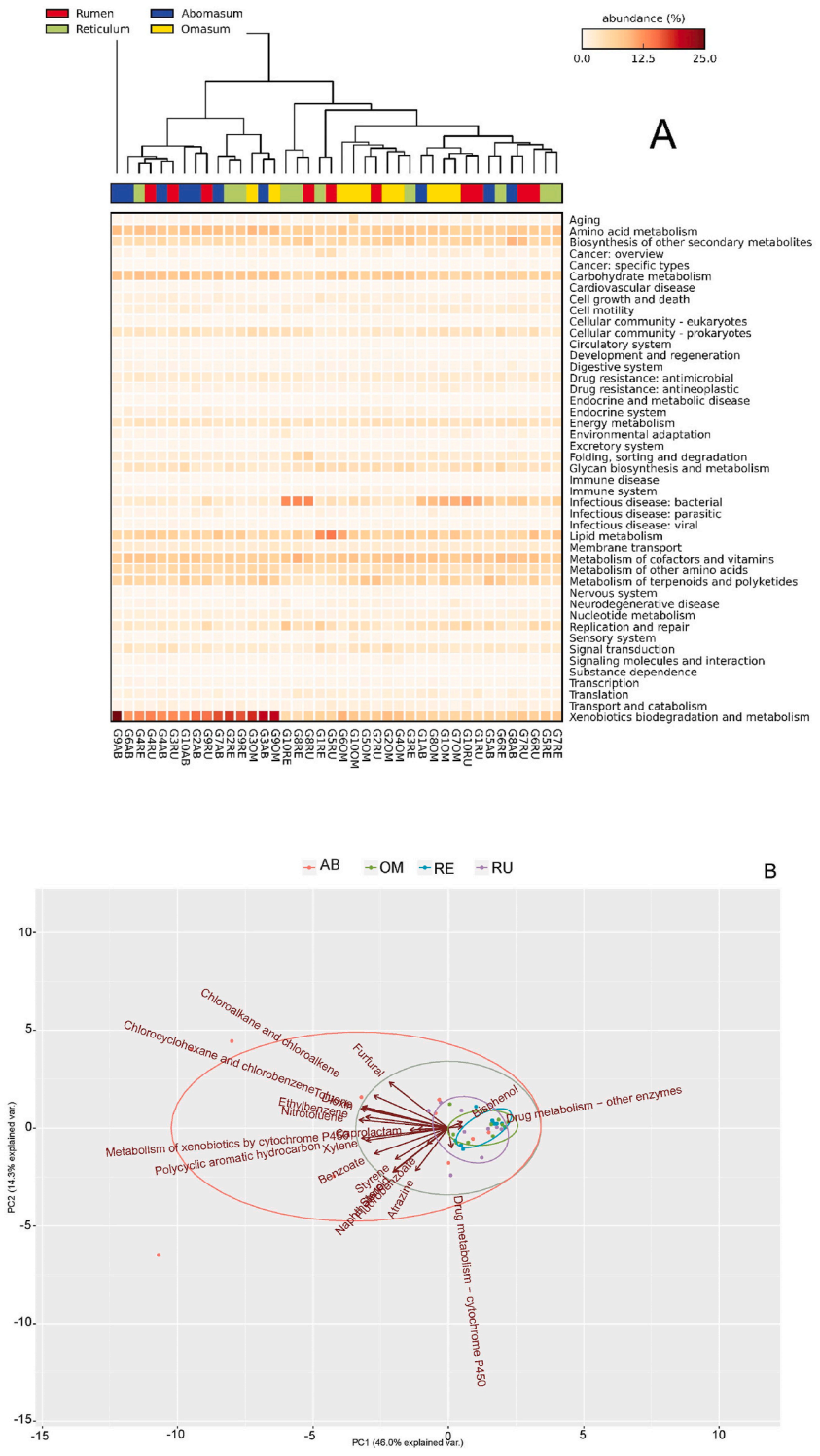

Fig. 4. **A**) Abundance of functional profiles of gene families in all the compartments based on the KEGG database. **B**) Principal Component Analysis (PCA) plot showing the distribution of various xenobiotics degradation pathways across the four compartments, Rumen (RU), Reticulum (RE), Omasum (OM), Abomasum (AB).

21.88 % and 23.92 %, respectively. The rumen exhibited the highest diversity of both genera, and this observation could be attributed to the unique physiological conditions of the rumen, which serves as the primary fermentation chamber in the goat's digestive system [37]. The rumen provides an anaerobic environment rich in fermentable substrates from plant materials, such as cellulose and hemicellulose, facilitating the proliferation of cellulolytic and fibrolytic microorganisms [6].

The reticulum was the second compartment in which both these genera were prevalent, *Proteus* accounting for 18.08 % and *Bacteroides* present at 15.74 %. The efficient degradation of feed in the rumen would not be possible without the cooperative role of the reticulum, which is located adjacent to the rumen and acts as a mixing chamber by facilitating the regurgitation and rumination of partially fermented feed, forming the cud [6,37]. This rumination process is crucial in breaking down large feed particles into smaller ones, significantly increasing the surface area available for microbial colonization in the rumen. As a result, the microbial population can efficiently ferment the feed, leading to enhanced nutrient availability for the host [37,44]. Furthermore, the reticulum's contractions play a pivotal role in particle size reduction, fiber processing, and uniform distribution of ingested feed among the microbial population. Its honeycomb-like structure aids in trapping large fibrous particles, providing extended retention time for microbial activity. This dynamic process allows the microorganisms to break down complex plant material further, ensuring optimal nutrient utilization from the feed [37,45]. Therefore, these results emphasize the intricate interplay between the rumen and reticulum inefficient feed degradation.

In contrast, the omasum exhibited a relatively lower prevalence of genera than the other three compartments but exhibited a notably higher abundance of unclassified genera (43.51 %) and *Prevotella* genus (2.12 %). The omasum is a filtration chamber where water and fine particles are absorbed before the ingesta progresses to the abomasum. The reduced abundance of genera in the omasum could be attributed to its role in fine particle retention and water absorption, leading to a less favorable environment for diverse microbial growth [37]. Meanwhile, the abomasum displayed a distinct microbial profile compared to the other compartments, with *Raoultella*, *Hafnia,* and *Klebsiella* among the top ten in this compartment. Even though *Proteus* and *Bacteroides* were dominant, a significant decrease was observed, and they were present in lower quantities at 13.81 % and 13.22 %, respectively. A relatively higher abundance of *Enterococcus* (10.7 %), *Clostridium* (5.8 %), and *Escherichia* (4.54 %) genera were observed in the abomasum and were less prevalent in the other three compartments. This observation could be due to their ability to cope under lower pH conditions like the ones reported in the abomasum [40]. While the other observed genera are similar to previous research [33,40,41], identifying the *Proteus* genus as a prevalent genus in this study presents a unique and potentially significant finding. Sequences under 0.5 % were derived from archaeal communities, representing them in lower quantities than bacterial communities. A similar study also reported low observed quantities of archaeal communities, which suggested their low levels of participation in the degradation of polysaccharides in the GIT [33].

The following sections explore the putative functional roles of these microbial communities in each compartment concerning the pathways involved and the production of CAZymes.

3.3. Functional profiles of whole metagenomes of each compartment

Building on the preceding discussions on diversity and taxonomy, the functional profiles of the whole metagenomes within each compartment in the context of significant gene families will be discussed. The functional profiles of the microbial population represented in each compartment were predicted using the KEGG database. The heatmap (Fig. 4A) depicts the all the gene families predicted for each class including the metabolic pathways and their abundance in various compartments. At level 1 KEGG, more than 65 % of the predicted genes were found to be involved in metabolism. The most prevalent gene families from level 2 KEGG, including xenobiotic biodegradation and metabolism (12 %), metabolism of co-factors and vitamins (9 %), amino acid (9 %), carbohydrate (7 %), and lipid metabolism (7 %) were discovered. According to the findings, the quantity of these various functional classes varied greatly throughout the compartments, with some dem onstrating greater dominance than others (Figure S2).

Compared to other pathways, the number of genes associated with xenobiotic biodegradation and metabolism was relatively high in the abomasum compartment. The xenobiotics biodegradation and metabolism pathway in the KEGG database provides information on the enzymes and metabolic pathways involved in the biodegradation and metabolism of xenobiotic compounds, including environmental pollutants, drugs, and other foreign compounds, which further highlights the adaptability and complexity of the microbiota in these compartments. Xenobiotics are foreign substances not naturally produced or expected to be present in an organism's body [46].

Certain compounds can be regarded as xenobiotics in plant cell walls, as they may be recalcitrant or difficult to metabolize without the assistance of specific microbial communities [9,17]. Lignin, a complex polymer that provides rigidity to the cell walls and its derivatives, can be considered as such a compound due to its recalcitrant complex structure, and requires lignolytic enzymes produced by certain microbial taxa for it to be broken down [9,17,18]. Furthermore, phyla Firmicutes, Bacillota, and Proteobacteria have been reported to produce lignolytic enzymes, including lignin peroxidases, laccase, and manganese peroxidase [47]. Thus, the prevalence of Firmicutes and Proteobacteria, particularly in the omasum and abomasum compartments, might be attributed to their involvement in lignin degradation, assisting the host in accessing the nutrients encapsulated within plant cell walls. The xenobiotic pathways in this study related to lignin degradation were toluene, ethylbenzene, xylene, styrene, and polycyclic aromatic hydrocarbons, among others [48].

Hemicellulose is another plant cell wall compound that can be regarded as a xenobiotic, and the furfural degradation pathway was detected in this study due to its degradation (Fig. 4B). Pathways related to the degradation of environmental pollutants and industrial chemicals, such as atrazine, chloroalkanes, and chloroalkenes, show significant activity across all compartments but are particularly abundant in the abomasum (Fig. 4B). This suggests that the abomasum plays a crucial role in the detoxification and breakdown of

ingested toxic compounds into less harmful metabolites. Furthermore, pathways associated with the metabolism of pharmaceuticals and drugs, including cytochrome P450-mediated metabolism, exhibit varying levels of activity across compartments, with some showing prominent abundance in the abomasum. This indicates that the abomasum is actively involved in processing and detoxifying ingested medications and pharmaceutical compounds, converting them into less toxic forms. Moreover, the presence of pathways involved in the degradation of industrial chemicals like caprolactam, styrene, and xylene in the abomasum highlights the potential exposure of ruminants to these compounds through their diet. The significant activity of these pathways highlights the importance of the abomasum in handling and metabolizing xenobiotic compounds, ultimately converting them into less harmful substances.

Interestingly, the high abundance of xenobiotic degradation and metabolism across all compartments in this study is inconsistent with other studies conducted on goats [42,48], and ruminants, including dairy cows [41]. These observations present a unique and intriguing perspective within the context of existing research by offering an opportunity to explore new dimensions of xenobiotic interactions. The involvement of the microbial community within the compartment in xenobiotic degradation and metabolism, as observed in our study, presents thought-provoking insight from established findings in this field. While existing research may not align with our results, it is crucial to consider the potential influences of various factors that might contribute to this disparity. Environmental factors, such as diet and exposure to different browse, may play a pivotal role in shaping the metabolic responses observed in our study [40].

Likely, specific microbial populations residing within the goat's GIT could also contribute to xenobiotic metabolism, potentially introducing a previously unexplored dimension to this intricate process. Unlike in previous research studies, which were dominated by Firmicutes and Bacterioidota [33,40], the GIT samples in this study were dominated by the presence of the *Proteus* genus of the Proteobacteria phylum which primarily consists of bacterial strains suspected for their involvement in pollutant degradation and metabolism [49,50]. They have been reported to possess mechanisms that enable them to break down and utilize complex organic compounds, including hydrocarbons [51]. These properties make them essential contributors to bioremediation and environmental clean-up processes [49,50]. While our findings may seem different from prior research, they lead to new avenues of research and highlight the need for a more comprehensive understanding of species-specific and contextual influences on xenobiotic degradation and metabolism. Therefore, elucidating the underlying mechanisms driving this phenomenon under these findings should be warranted to establish a holistic perspective on the complex interplay between the *Proteus* genus and xenobiotic degradation and metabolism.

The carbohydrate metabolic pathway observed was also relatively abundant across all samples. This pathway is significant to the goat's GIT microbiome due to its involvement in the fermentation of complex carbohydrates into simple sugars that microbial communities can consume for energy and replenishment in the GIT. The number of carbohydrate metabolism pathways detected in all compartments showed that the microbial community was well adapted to plant cell wall degradation and absorption. Furthermore, all compartments had abundant amino acid and lipid metabolism. This was also a crucial observation because amino acids are required for protein synthesis and play a role in numerous physiological processes. These findings were similar to studies conducted on other goats [33,40].

3.4. Classification of Metagenome-Assembled Genomes and CAZymes identification

The reticulum and omasum exhibited higher MAGs representation of 32 % and 29 %, respectively, with the abomasum having the lowest (18 %). See table S2 and Fig. 5. At the genus level, MAGs from *Bacteroides* were consistently present across all compartments, suggesting their importance in GIT degradation and metabolism. *Proteus* MAGs were less abundant in the abomasum. Meanwhile, *Enterococcus_B* and *Escherichia* MAGs exhibited compartment-specific variations, with higher counts in the abomasum. Furthermore, *Enterococcus_C* and *Peptostreptococcus* were only present in the first three compartments, and none were observed in the abomasum. *Bifidobacterium*, *Fibrobacter*, and *Butyrivibrio* MAGs appeared to be relatively more abundant in the omasum, potentially indicating their involvement in later digestion and nutrient absorption stages.

The dbCAN2 prediction search revealed various CAZyme classes, including glycoside hydrolases (GHs), glycosyl transferases (GTs), carbohydrate esterases (CEs), carbohydrate-binding modules (CBMs), polysaccharide lyases (PLs), and auxiliary activities (AAs). All

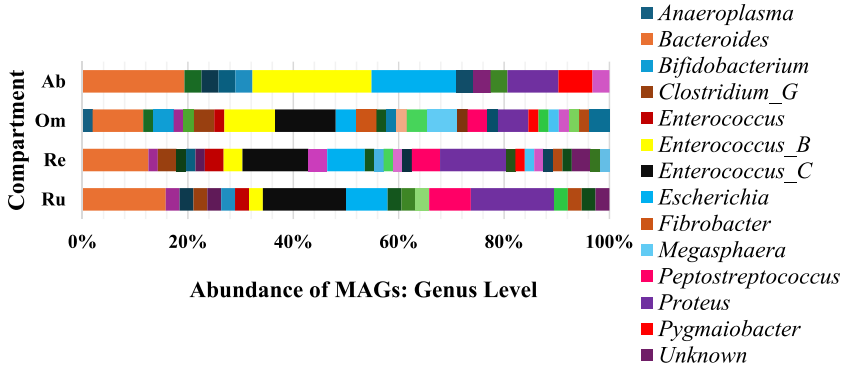

Fig. 5. The abundance (%) of MAGs represented in each compartment at genus level.

the hits are profiled in supplementary table S4. The search resulted in a total of 32 455 hits, of which 79 % encoded for GHs (25 551 hits), which are a large family of enzymes that are responsible for the hydrolysis of glycosidic bonds between carbohydrates or between a carbohydrate and a non-carbohydrate moiety. These enzymes are involved in the degradation of various carbohydrates, including cellulose, hemicellulose, and pectin, which are the major components of plant cell walls [52,53]. GTs (3144 hits), PLs (1896 hits), and CEs (1242 hits) accounted for 10 %, 6 %, and 4 % of the hits, respectively (Fig. 6A). GTs transfer sugar moieties from activated donor molecules to acceptor molecules, forming glycosidic bonds [53], while PLs facilitate the depolymerization of anionic polysaccharide chains via a β-elimination mechanism [54]. CEs play an essential role in the breakdown of lignocellulosic biomass by cleaving ester bonds between lignin and hemicellulose or pectin, making the cellulose and hemicellulose more accessible to other enzymes [52]. CBMs and AAs domains accounted for only 1 % of the hits. CBMs domains bind specifically to carbohydrate substrates and allow CAZymes to target and degrade or modify carbohydrates, while AAs are involved in the breakdown and modification of complex carbohydrate structures such as lignocellulose, chitin, and hemicellulose [53].

Previous studies have also investigated the microbiome of ruminants for the presence of CAZymes responsible for the efficient degradation of lignocellulosic biomass. An analysis of the camel rumen microbiome found that among the CAZymes that were detected, GHs were the most abundant, accounting for approximately 49.5 %, followed by GTs at 30.8 %, and the other CAZymes accounted for the remaining 19.61 % [26]. Similarly, another study of the buffalo rumen metagenome identified GHs as the most abundant, followed by GTs [43]. The CAZymes were relatively higher in the reticulum and omasum compartments (Fig. 6B), possibly due to the MAGs diversity observed.

Table S2shows the number of hits for various GHs, with the GH13 and GH43 families having the highest hits at 7300 and 6590, respectively. The reticulum had higher hits in both GH13 and GH43 families, accounting for 2278 and 2056 hits, respectively. The abomasum had the least amount of hits in both. GH13 enzymes are α-amylases, branching enzymes, and α-glucosidases, which are responsible for the hydrolysis of α-1,4-glycosidic bonds in starch and related polysaccharides. GH43 enzymes include xylanase, arabinases, β-1,3- xylosidase, and β-xylosidase, which hydrolyze xylan, a significant hemicellulose component. The high number of hits for these two families can provide a snapshot of the diet of these goats. Other GHs with a high number of hits include GH2 (885 hits), GH3 (883 hits), GH5 (804 hits), and GH30 (522 hits). GH2 enzymes are β-galactosidases, which break down lactose and related complex carbohydrates. GH3 enzymes are β-glucosidases, which break down cellobiose and related carbohydrates; meanwhile, GH5 enzymes are endo-β-1,4-glucanases, which are involved in the degradation of cellulose. GH30 enzymes are also involved in cellulose degradation, specifically cleaving β-1,4-glycosidic bonds in the backbone of cellulose [52].

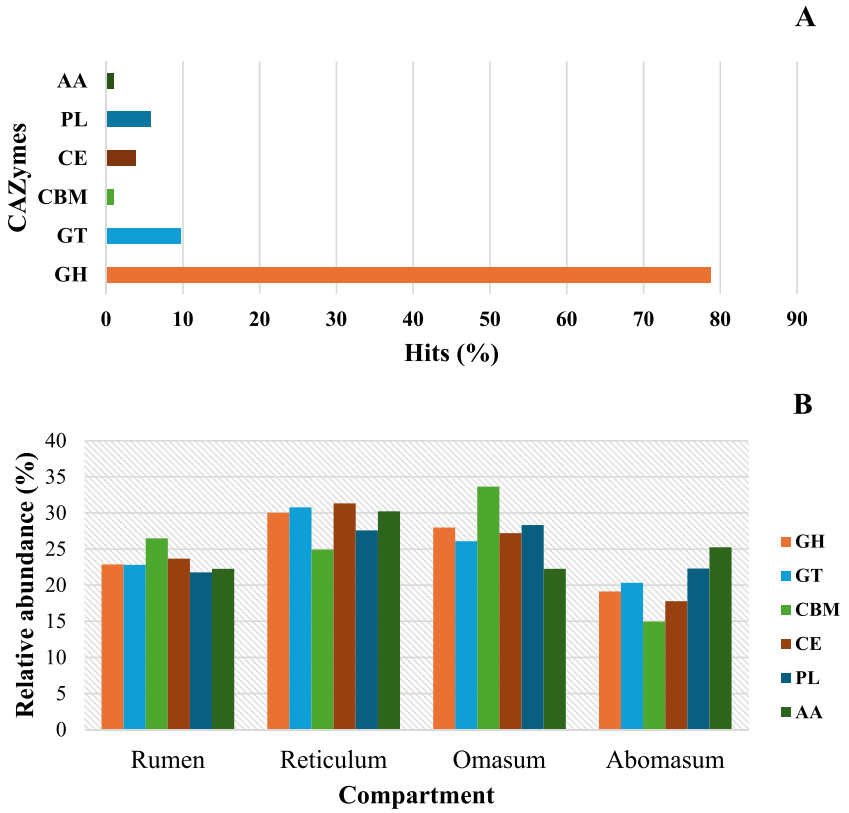

Fig. 6. A) The abundance (%) and prevalence of CAZymes across from all four compartments; glycoside hydrolases (GHs), glycosyl transferases (GT), carbohydrate esterases (CEs), carbohydrate-binding modules (CBMs), polysaccharide lyases (PLs), and auxiliary activities (AAs) **B)** The distribution of CAZymes hits in all compartments.

The presence of various GTs in the goat gut microbiome was also indicative of the diversity of microorganisms present in the digestive system of these goats. GTs are responsible for transferring sugar moieties between molecules, resulting in the formation of glycosidic bonds [52]. However, the ability of GTs to act on lignocellulosic biomass is still under research. While some studies suggest that GTs may not play a significant role in the degradation of lignocellulosic biomass [52], it has been reported that GTs may be involved in modifying hemicelluloses [55,56]. Therefore, depending on the microbial source, GTs can modify the hemicellulose by attaching a sugar molecule or modifying an existing sugar molecule, thereby altering the physical and chemical properties of hemicellulose, potentially making it more accessible to CAZymes involved in the degradation of lignocellulosic biomass. Accounting for 362 hits, PL11 was the most abundant family among the known PLs, followed by PL1 and PL8 with 314 and 277 hits, respectively. PL4, PL27, and PL31, on the other hand, had the fewest hits. Based on the number of matches for each CE, CE1, CE9, and CE12, they appear to be the most common in the sample. CE8 and CE4 also alter glycan structures, critical for cell signaling and recognition. CE7, which is engaged in glycan structure synthesis, had fewer hits.

Few hits for CE11, CE6, CE19, CE2, CE15, CE17, CE5, and CE14 indicated that they were less common in the samples. CBM67 was

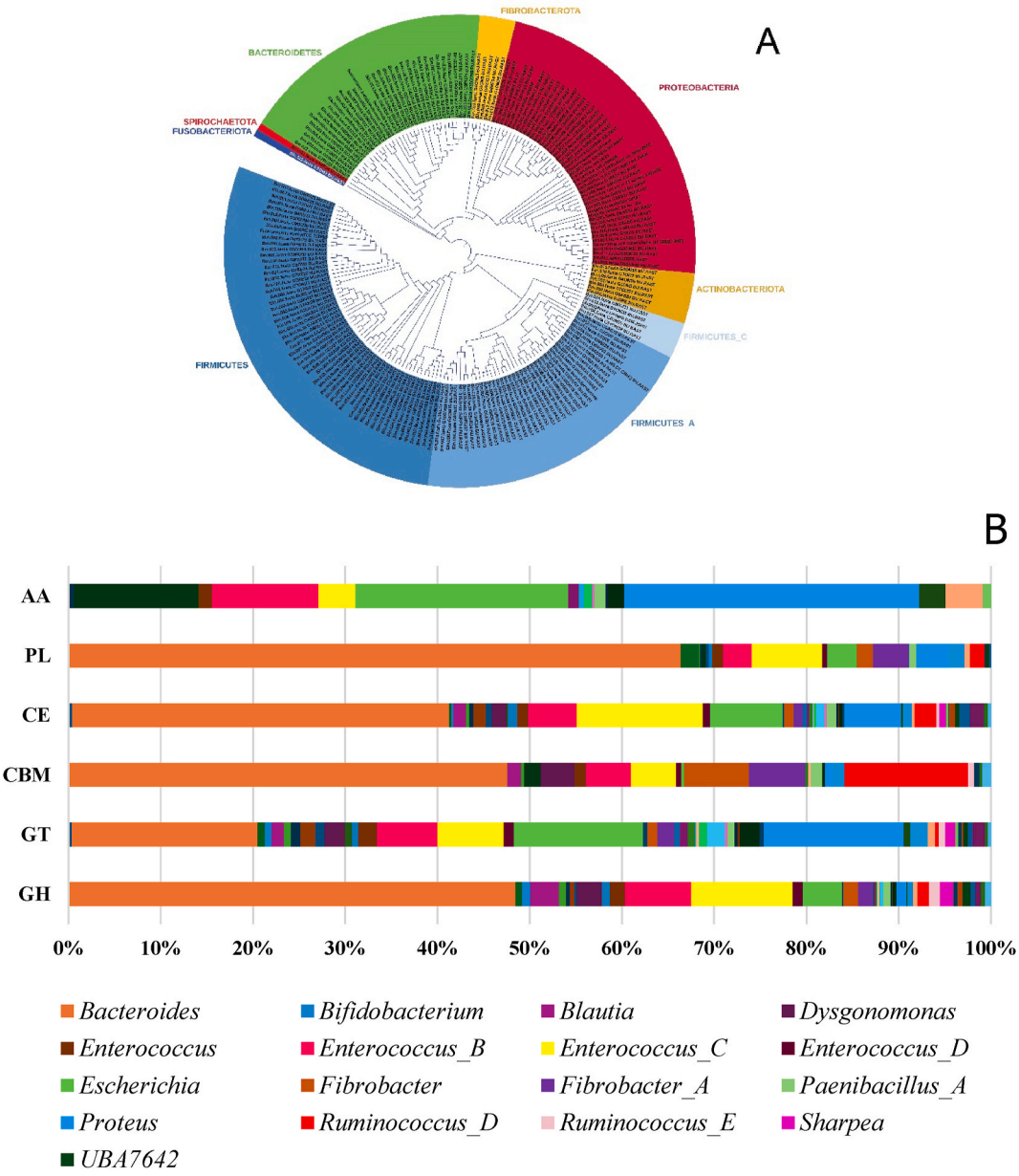

Fig. 7. A) Phylogenetic tree showing relativity of the taxa responsible for secreting CAZymes [29]. **B)** The bar chart shows the abundance of different CAZyme families produced by each genus. GH, GT, CBM, CE, PL, and AA represent glycoside hydrolases, glycosyltransferases, carbohydrate-binding modules, carbohydrate esterases, polysaccharide lyases, and auxiliary activities, respectively.

the most common family, with 105 hits, followed by CBM34 (45 hits), CBM6 (40 hits), and CBM37 (32 hits) in the CBMs search results. CBMs are non-catalytic domains found in CAZymes that identify and bind carbohydrates in lignocellulosic biomass. CBMs are divided into families based on sequence similarity and carbohydrate-binding specificity. In this analysis, CBM67 was the most common domain. CBM67 is found in enzymes that degrade crystalline cellulose, and they have a high affinity for L-rhamnose, a sugar contained in the cell walls of various plants, particularly lignocellulosic biomass. A substantial number of CBM67 domains in the goat microbiome indicates that goats have a high potential for effective cellulose degradation [57].

AAs are proteins that do not belong to the CAZymes families but are required for the complete degradation of complex carbohydrates and organic compounds such as lignin. These lignin-modifying enzymes (LMEs) include lytic polysaccharide monooxygenases (LPMOs), carbohydrate esterases, and other accessory enzymes. The hits for AAs were relatively low compared to the different families of enzymes discussed earlier because AAs are not directly involved in the depolymerization of complex carbohydrates but instead assist in the degradation process by breaking down the non-carbohydrate components of these substrates or modifying the carbohydrate polymers to make them more accessible to other enzymes. AA3 was the most prevalent family, with 150 hits. The AA3 family includes several LPMOs that enhance cellulases and hemicellulases' activity by cleaving and modifying the cellulose and hemicellulose polymers [52,58]. Although most AA hits are from the reticulum, the abomasum had a relatively higher number of AA hits than the other two compartments.

The Firmicutes phylum was the most dominant group of microorganisms in the goats' GIT responsible for lignocellulosic biomass, with approximately 32.2 % coverage. This phylum contains many species that can efficiently degrade lignocellulose, including genera *Butyrivibrio*, *Ruminococcus,* and *Clostridium* [43]. These microorganisms produce a range of lignocellulolytic enzymes such as cellulases, hemicellulases, and ligninases, which break down the complex lignocellulosic biomass into simpler sugars that can be further utilized by the microorganisms as a source of energy [26]. Most of the hits in the GIT microbiome belonged to the genera *Enterococcus*, specifically *Enterococcus_B* and *Enterococcus_C*, and were primarily associated with the GH family of CAZymes.

Proteobacteria was the second most abundant phylum responsible to produce CAZymes with approximately 20 % coverage and are one of several members that have been shown to have a role in lignocellulose degradation. Members of the genera *Succinivibrio* can degrade lignocellulose to varying degrees through the secretion of various hydrolytic enzymes [59,60]. Similarly, Bacteroidetes accounted for approximately 17.2 % of the MAGs. They include several members reported to be associated with butyrate production in the rumen and are involved in the degradation of lignocellulosic biomass [61]. Members of the genera *Bacteroides* and *Prevotella* are known to produce a range of lignocellulolytic enzymes that help break down complex plant material [43]. Actinobacteriota and Fibrobacterota phyla accounted for 3.89 % and 2.78 % of the MAGs, respectively. Although they were less abundant than Firmicutes, Proteobacteria, and Bacteroidetes phyla, they are also known to play a vital role in lignocellulosic biomass degradation [43].

Firmicutes_C, Spirochaetota, and Fusobacteriota phyla, on the other hand, were identified in significantly lower percentages, at 2.2 %, 0.56 %, and 0.56 %, respectively. Although they were less prevalent, members of these phyla are also capable of lignocellulose degradation, producing a variety of enzymes that aid in the breakdown of complex plant material. In a previous study investigating the CAZymes from a buffalo metagenome fed with green and dry roughage, Bacteroidetes, Firmicutes, Proteobacteria, and Actinobacteria, in descending order, were found to be the most abundant phyla involved in the production of various CAZymes [43].

Out of 180 MAGs, about 14 amounted to 3938 genomes that could not be accurately identified as any known species (Table S2). Considering the high quality of these MAGs, this suggested that there were likely more unidentified and uncultured microbial species in goat GIT, which are not represented in existing reference databases. These observations are essential for understanding the complex microbial communities in the GIT and their role in lignocellulosic biomass degradation. The unassigned MAGs at the species level belonged to the genera *Anaeroplasma* (1), *Butyrivibrio* (1), *Enterococcus_C* (1), *Fusobacterium_A* (1), *JAAME01* (1), *Massilibacterium* (3), *Ruminococcus_E* (2), *Treponema_D* (1) and Unassigned genera (3). Furthermore, these findings suggested that there is still much to be discovered about the microbial population within the GIT of free-range goats and their ability to break down lignocellulosic biomass. A phylogenetic tree showing the relatedness of the taxa responsible for the production of CAZymes at the phylum level is shown in Fig. 7A.

Enterococcus_B, *Enterococcus_C*, *Escherichia*, and *Proteus* were found in all CAZyme families (Fig. 7B). The *Bacteroides* genus was found in all families. However, no hits for AAs were observed. *Proteus* is a genus prevalent in the GIT of goats and other ruminants such as cattle and sheep [49]. Although *Proteus* is considered an opportunistic pathogen, its presence tends to be responsible for a symbiotic association between different groups of bacteria and the host animal, including ruminants [49]. Few studies extensively highlight the role of the genus *Proteus* directly from the GIT, but it is suspected that *Proteus* bacteria in the GIT play an essential role in the breakdown of complex carbohydrates, such as cellulose and hemicellulose. This is due to *Proteus*'s ability to secrete a consortia of enzymes including cellulases and xylanases which are involved in the breakdown of complex carbohydrates into fermentable sugar molecules that can then be absorbed by the goat's digestive system and also produce ammonia, which is a crucial source of nitrogen for other microorganisms in the GIT [62]. Thus, the presence of the genus *Proteus* may prove essential in the ruminants' GIT due to its involvement in the degradation of the plant material. In addition, the chances of successfully isolating and characterizing various *Proteus* strains to contribute to scientific knowledge on lignocellulosic biomass degradation using these goats' microbiomes are significantly higher.

Bacteroides and *Enterococcus* are also common commensal bacteria found in the GIT. While the dominance of specific genera suggests their significant presence within the goat's GIT, it is important to note that other bacterial groups, though less abundant, may still play a critical role in the overall function of the GIT. *Bacteroides* and other groups, such as *Clostridia*, *Ruminococcus,* and *Clostridium,* have been reported to produce fermentative hydrogen in the rumen [26]. Meanwhile, *Enterococcus* genera have been reported as one of the primary lactic acid producers and utilized as probiotics. In addition, *Enterococcus* strains are known to be resistant to bile salts and gastric juices [28] and can secrete crucial enzymes, including lipase, proteases, peptidases, and esterases [63], and can restrict the

growth of harmful microorganisms [64]. Mansour et al. (2018) used three *Enterococcus* strains to inhibit the *Clostridium difficile* in Mice to control *C. difficile* infection. *Enterococcus faecium* is beneficial for supporting the function of lactate-fermenting microorganisms and promoting the development of rumen microorganisms. Accordingly, this may increase the supply of glucogenic propionate energy for the host [65]. Li et al. (2018) reported two *Enterococcus* strains, *E. faecalis JF85,* and *E. faecium Y83,* from the Tibetan Yak rumen, with the potential to degrade cellulose [66]. These findings suggest that *Enterococcus* may play various roles in the GIT, including the efficiency of lignocellulosic biomass degradation through the production of VFAs, which eventually replenish the nutritional balance in the overall GIT feed flow. These findings suggest a diverse range in the microbial population within the goat GIT capable of breaking down lignocellulose, further highlighting the complex and efficient interaction nature of microbial degradation of lignocellulosic biomass in the goat's GIT.

Acknowledging the limitations of our study is crucial for providing a comprehensive understanding of our research findings and guiding future investigations. One limitation is that our focus was solely on the metagenomics of the ruminal samples without concurrent physicochemical, elemental, or nutrient analyses. While metagenomics information provides valuable insights into the microbial composition and potential capabilities, integrating additional analyses could offer a more holistic understanding of the ruminal environment and its metabolic activity. Future studies should consider incorporating these analyses to complement microbial profiling data and provide a more comprehensive characterization of the GIT ecosystem. Furthermore, exploring correlations between microbial composition and environmental factors, such as pH, temperature, moisture content, and nutrient availability, through physicochemical and nutrient analyses would deepen our understanding of microbial dynamics and adaptations.

4. Conclusion

In conclusion, this study has illuminated the complex microbial diversity and functional profiles within a goat's forestomach, particularly in producing CAZymes. The distinct variations in species composition between compartments, coupled with the high richness and diversity observed in the omasum, may emphasize the importance of each compartment in the GIT. Notably, the higher prevalence of the *Proteus* genus observed in this study adds to scientific knowledge where gaps exist in how this genus interacts with other microorganisms in a natural environment. The highlighted gene families associated with xenobiotic degradation, amino acid, lipid, and carbohydrate metabolism, particularly xenobiotic degradation and metabolism, hold great potential for advancing bioremediation strategies in environmental biotechnology.

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Data availability

Data will be made available on request.

CRediT authorship contribution statement

Kgodiso J. Rabapane: Writing – original draft, Validation, Software, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Tonderayi S. Matambo:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

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

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Appendix ASupplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e37508.

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