

MICROBIAL ECOLOGY IN EXTREME ENVIRONMENTS: METAGENOMIC INSIGHTS INTO THE GUT MICROBIOME OF THE INDIAN WILD ASS (*Equus hemionus khur*)

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<https://doi.org/10.55251/jmbfs.12159>

ARTICLE INFO

Received 13. 12. 2025

Revised 9. 6. 2026

Accepted 24. 7. 2026

Published 1. 8. 2026

Regular article



ABSTRACT

The Indian wild ass (*Equus hemionus khur*), also called the Ghudkhur or Khur, it is a subspecies of the onager native to Southern Asia. The Indian wild ass inhabits the highly saline desert ecosystem of the Little Rann of Kutch (LRK) and its surrounding area in Gujarat, India. The Little Rann of Kutch is characterized by its saline desert environment, which provides a suitable habitat for these animals. It is a very harsh condition to survive in such a polyextreme environment. The gut microbiome plays a crucial role in digestion, nutrient absorption, and overall host health and adaptation. Due to the unique adaptation of ass and limited data available on the gut microbiota of the Indian wild ass, this study presents a comprehensive metagenomic analysis of the gut microbiome of the Indian wild ass (*Equus hemionus khur*), offering novel insights into microbial adaptations in extreme environments. Using 16S rDNA sequencing and advanced bioinformatics, we characterized a diverse microbial community comprising 32 phyla, 58 classes, 130 orders, 253 families, 452 genera, and 627 species. Firmicutes (39.03%), Bacteroidetes (23.24%), and Proteobacteria (13.66%) emerged as the dominant phyla, with a notable presence of halophilic and halotolerant species reflecting adaptations to the saline desert habitat. Functional analysis indicated the Indian wild ass gut microbiome showed diverse metabolic capabilities. Notably, we identified significant potential for plant growth promotion and stress mitigation, suggesting complex host-microbe-environment interactions. The presence of autotrophic microbes (17.1%) and adaptations to various temperatures, highlight the community's resilience in harsh conditions. This study not only enhances our understanding of microbial ecology in extreme environments but also provides insights into host-microbe interactions in endangered species and also presents a valuable resource for biotechnological innovations in environmental management, sustainable agriculture, and industrial enzyme production. The findings open new avenues for research in microbial genetics, metabolism, and systems biology, with potential implications for conservation microbiology and the study of microbial adaptations to environmental stressors.

Keywords: *Equus hemionus khur*, gut microbiome, microbial diversity, microorganisms of extreme habitats, metagenomics of saline habitats

INTRODUCTION

The Indian wild ass (*Equus hemionus khur*), locally known as khur, ghodkhad, or ghorkhar, is a charismatic equid subspecies endemic to the Little Rann of Kutch (LRK) in Gujarat, India. This unique ecosystem, characterized by its arid landscape and saline mudflats, serves as the last refuge for this endangered subspecies (Barman *et al.*, 2022; Gee, 1963; Raval, 1988; Rina *et al.*, 2009; Singh, 2000). Historically, the *Equus hemionus* was widespread across southern Pakistan, Afghanistan, and southeast Iran. Still, the Indian wild ass subspecies (*Equus hemionus khur*) are now restricted within the Indian wild ass Sanctuary (established in 1973 to protect the dwindling population) and nearby areas. Conservation efforts have shown promising results, with population numbers rebounding from a critical low of 362 individuals in 1967 to 7672 as of the 10th Wild Ass Population Estimation (WAPE) 2024 conducted by the Gujarat Forest Department, Government of Gujarat, India. This recovery, while encouraging, underscores the importance of sustained academic research and conservation efforts to ensure the long-term sustainability of the species (Raval, 1988; Singh, 2000). However, the Indian wild ass continues to face ongoing challenges, including habitat loss, human-wildlife conflict, and potential genetic bottlenecks due to its historically small population size (Khaire *et al.*, 2017; Prasad *et al.*, 1994; Singh & Soni, 1999).

The dietary adaptations of the Indian wild ass are particularly intriguing. These animals primarily consume a mix of salt-tolerant grasses and sedges from the Poaceae, Chenopodiaceae, and Fabaceae families. This specialized diet, along with the unique environmental pressures of the LRK, suggests that the Indian wild ass may harbor a distinctive gut microbiome that facilitates its survival in this challenging habitat (Pokharia *et al.*, 2022; Raval, 1988; Suthar *et al.*, 2018). The gut microbiome plays a pivotal role in influencing the overall health, immunity, and metabolism of mammals, including equids, and is significantly affected by environmental variables, particularly diet (Chaucheyras-Durand *et al.*, 2022; Paul *et al.*, 2022; Y. Zhu *et al.*, 2021; Dapa *et al.*, 2024; Barathan *et al.*, 2024).

The Indian wild ass has evolved to thrive in the saline ecosystem of the Little Rann of Kutch, characterized by high salt concentrations in both soil and vegetation. Given the established link between environmental factors and gut microbiome composition, the gut microbiome of *Equus hemionus khur* may contain a higher proportion of halophilic or halotolerant microbial species (Rina *et al.*, 2009; Khunt *et al.*, 2011), reflecting adaptations to its high-salt habitat. While further investigation is required to elucidate the potential adaptive mechanisms of the Indian wild ass's gut microbiome in response to its saline ecosystem, the specific composition and function of this microbiome remain largely unexplored. This study aims to provide a comprehensive characterization of the Indian wild ass gut microbiome using 16S rDNA metagenomic sequencing. The Sequencing technologies and bioinformatics techniques provided a complete view of microbial diversity and functional potential, which enhanced our understanding of the gut microbiome's role in health and disease (Gao *et al.*, 2023). While extensive research has been conducted on the gut microbiomes of various mammalian species, including domesticated equids, a significant gap still exists in our understanding of microbial communities in wild animals inhabiting extreme environments.

The Indian wild ass (*Equus hemionus khur*), native to the saline desert of the Little Rann of Kutch, presents a unique opportunity to explore microbial adaptations to harsh conditions. Previous studies have highlighted the importance of gut microbiomes in host health, nutrition, and environmental adaptation, but the specific microbial strategies employed in saline desert ecosystems still need to be explored. Moreover, the potential biotechnological applications of microorganisms adapted to such extreme conditions have yet to be thoroughly investigated. This study will therefore fill these knowledge gaps by carrying out an integrative metagenomic analysis on the gut microbiome of the Indian wild ass. We seek to not only enhance our understanding of host-microbe interactions in extreme environments but also uncover novel microbial resources with potential applications in areas such as bioremediation, salt-tolerant enzyme production, or other biotechnological innovations, conservation, and environmental management.

The findings from this research will contribute to the growing body of knowledge in microbial ecology while offering insights into the adaptive mechanisms of both host and microbes in challenging habitats.

MATERIAL AND METHODS

Sample Collection

Fresh fecal samples were collected non-invasively from a free-ranging Indian wild ass (*Equus hemionus khur*) at Little Rann of Kutch (LRK), Gujarat, India (Figure 1). The collected samples were immediately stored in a mobile refrigerator (4°C) before being transported to the laboratory. The composite samples were prepared from six areas, with 5 to 6 samples for each composite. These were then mixed to create a single composite sample.

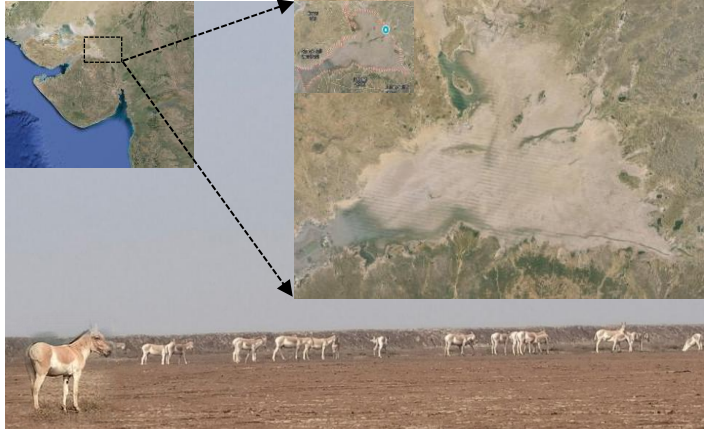


Figure 1 The zoomed-in satellite map view of Little Rann of Kutch (LRK), a salt marsh in Gujarat, India. At the same time, the main picture provides a ground-level view of a group of free-ranging Indian wild asses in their natural habitat at Little Rann of Kutch (LRK), Gujarat, India.

DNA Extraction and PCR Amplification

According to the manufacturer's instructions, DNA was extracted from the single composite sample using the HiPurA™ stool DNA purification kit (MB544, Himedia). DNA quality was evaluated using 1% (w/v) agarose gel electrophoresis, while its quantity was determined by assessing the A260/280 ratio with a NanoDrop spectrophotometer. The V3-V4 hypervariable region of the 16S rDNA gene was amplified using PCR with universal primers 341F (CCTACGGGNGGCWGCAG) and 805R (GACTACHVGGGTATCTAATCC). The PCR reaction mixture (25 µl)

contained ten ng of metagenomic DNA, one µM each of forward and reverse primers, and 2x KAPA Hi-Fi HotStart ReadyMix.

Library Preparation and Metagenomic Sequencing

The sequencing library was prepared using the Nextera XT DNA library preparation kit according to the manufacturer's instructions. 16S rDNA metagenomic sequencing was performed using an Illumina MiSeq sequencer at Anand Agriculture University (AAU), Anand, Gujarat, India. The sequence was deposited in the NCBI Sequence Read Archive under the Bio Project PRJNA1170020.

16s rDNA Metagenomic Sequence Analysis

The forward and reverse paired-end FASTQ data obtained from sequencing were merged using PEAR software (v0.9.6.4) a tool designed for assembling overlapping paired-end reads. Quality control of the merged sequences was performed using FASTQC (v0.74) (Andrews, S. (2017)). Taxonomic and functional profiling were analyzed using standard bioinformatics software. Taxonomic analyses of metagenomic sequencing data were performed using Kraken2 software (v2.1.3) (Wood & Salzberg, 2014) which assigns taxonomic labels using exact k-mer matches against a reference database. The taxonomic results were then visualized and explored using Pavian software (v1.0) (Breitwieser & Salzberg, 2020). The diversity indices were measured by PAST 4.03 (Paleontological Statistics) (Hammer et al., 2001) software. A taxonomic to phenotypic mapping was performed using METAGENassist software (Arndt et al., 2012). The Metagenomic analysis of plant growth-promoting traits (Patz et al., 2024) was done by MEGAN6 (MEtaGenome Analyzer) (v6.25.10) (Huson et al., 2007). Predictive functional metagenomic analysis was performed using the global mapper module of iVikodak software (Nagpal et al., 2016) which utilizes the KEGG Orthology (KO) database for functional annotation.

Data Acquisition

In this study, 18 available Kutch saline desert soil metagenome sequences were retrieved from the NCBI SRA database for use in discussion purpose (SRR numbers- SRR975526, SRR1042602, SRR953534, SRR953542, SRR975530, SRR1246236, SRR1246237, SRR953533, SRR901749, SRR975533, SRR975534, SRR1246239, SRR1245949, SRR975528, SRR953529, SRR1246238, SRR901747, SRR953559).

RESULTS AND DISCUSSION

Sequence Quality and Diversity Indices

Sequencing generated paired-end reads with forward (R1) and reverse (R2) sequences, which were processed and analyzed. Initially, we used PEAR (Paired-End reAd mergeR) software (J. Zhang et al., 2014) to merge the forward and reverse sequences. This process resulted in a single FASTQ file.

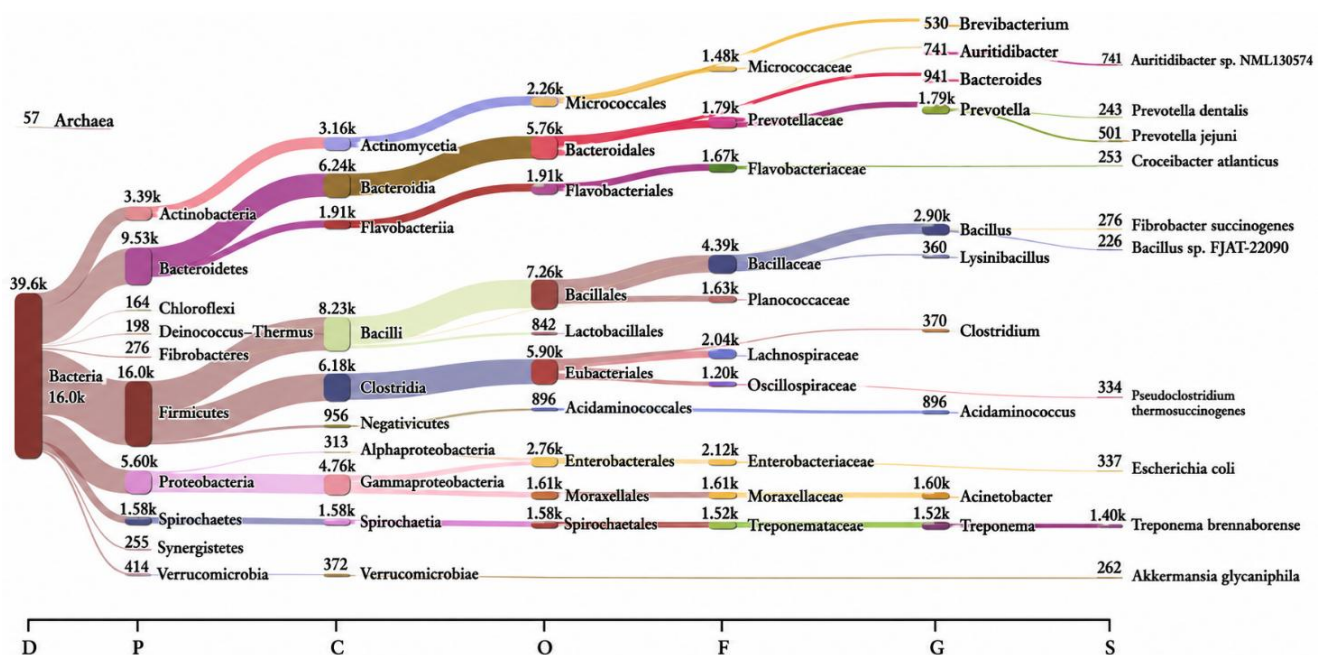


Figure 2 Taxonomic profile of Gut Microbiomes in Indian Wild Ass. The different sectors in the Sankey plot represent different taxonomic categories (from domain to species). Each sector color-coded and labeled represents the top ten microbial species. The numerical values correspond to the specific abundance of microbial species.

The sequencing data quality assessment revealed high overall quality, with no sequences identified as poor quality. The sample provided 40,991 reads, comprising 20.5 Mbp of sequence data. The reads ranged from 160 to 502 base pairs, with an average GC content of 55%. Of the total reads, 96.92% were classified by the Kraken2 taxonomic classification system. The Indian wild ass shows greater species richness (Taxa_S = 965) and lower dominance, indicating a more even distribution of species within its gut microbiome. The high Simpson's Diversity Index (0.980) suggests greater diversity and low dominance by any single species. Similarly, the elevated Shannon-Wiener Index (4.671) reflects both high richness and evenness. The high values of Fisher's Alpha (151.171) and Chao1 Index (1068.357) further support the presence of a richer and more diverse microbial community. These diversity indices indicate a higher microbial diversity and even distribution in the Indian wild asses' gut microbiome. This variation in microbial diversity could be attributed to differences in diet patterns, host phylogeny, evolutionary history, and environmental factors, underscoring the necessity for specific conservation strategies for each population.

Taxonomic Analysis

The gut microbiome taxonomic analysis revealed the number of distinct taxonomic ranks identified for each species. The Indian wild ass sample yielded classifications for 32 phyla, 58 classes, 130 orders, 253 families, 452 genera, and 627 species. The Indian wild ass demonstrated greater diversity at the lower taxonomic (family, genus, and species) levels.

The gut microbiome of the Indian wild ass (*Equus hemionus khur*) is predominantly composed of bacteria (99.86%), with a minor presence of archaea (0.14%). The taxonomic profile revealed a diverse bacterial community with taxonomic levels ranging from phylum to species. The phylum-level analysis identified 32 phyla, with Firmicutes emerging as the dominant phylum (39.03%), followed by Bacteroidetes (23.24%), Proteobacteria (13.66%), and Actinobacteria (8.27%). Other top ten identified phyla include Spirochaetes, Verrucomicrobia, Synergistetes, Fibrobacter, Deinococcus-Thermus, and Chloroflexi, in lower abundance. The phylum-level taxonomic profile of Indian wild ass is consistent with findings in other equine species such as Asiatic wild ass (Tang et al., 2022), Tibetan wild ass (Gao et al., 2020) and Mongolian wild ass (Edwards et al., 2020). Firmicutes play a crucial role in the degradation of dietary fibers, butyrate

production, β -mannan metabolism, bile acid transformation, host homeostasis and immune regulation, and interbacterial antagonism (La Rosa et al., 2019; Lee et al., 2022; Leth et al., 2023; Sun et al., 2023). Actinobacteria significantly enhance the digestive capabilities of herbivores by breaking down complex plant materials, detoxifying harmful compounds, and contributing to the host's nutritional needs, thereby playing a crucial role in the digestive ecology of herbivorous animals (Anteneh & Franco, 2017; Dearing & Kohl, 2017; Kohl et al., 2014; Latha & Dhanasekaran, 2013). Deinococcus-Thermus bacteria, typically associated with extreme environments such as hot springs and arid deserts, are present in the Indian wild ass and other equine gut microbiomes (horse (Gilroy et al., 2022) and Tibetan wild ass (H. Zhang et al., 2020)). This can be attributed to their robust enzymatic machinery and stress-resistant properties, which allow them to survive and function in the dynamic and harsh conditions of the gut.

The class Clostridia (order Eubacteriales) and Bacilli (orders Bacillales and Lactobacillales) are predominant within Firmicutes. Bacteroidia (order Bacteroidales) is the major class within Bacteroidetes. Gammaproteobacteria and Alphaproteobacteria are the main classes of Proteobacteria, and Actinobacteria is represented primarily by the class Actinomycetia. At the family level high abundance of the Bacillaceae (10.70%) was observed, followed by Enterobacteriaceae (5.17%) and Lachnospiraceae (4.97%). The comparative analysis of bacterial diversity at the family level within Kutch soil indicated a pronounced prevalence of the Bacillaceae family in 61% of the examined soil samples (Figure 3). Rojas et al. (2021) analyzed the high association of Enterobacteriaceae and Bacillaceae in the African herbivore Masai giraffe gut microbiome, further supporting the potential ecological significance of these families in herbivorous gut microbiomes. Several studies reported an abundance of Lachnospiraceae and Prevotellaceae in the gut microbiome of humans (Gosalbes et al., 2011), cows, deer, and sheep (Kittelmann et al., 2013), shetland pony, Mongolian wild ass, plain zebra (Bao et al., 2022), Carneddau ponies (Antwis et al., 2018). Lachnospiraceae's role in the degradation of complex plant polysaccharides, fiber fermentation, and short-chain fatty acids (SCFA) production supports the herbivorous diet, while the presence of Enterobacteriaceae plays multifaceted roles in the gut microbiome of herbivores, contributing to both beneficial functions and disease states, influenced by a variety of environmental and host factors (Baldelli et al., 2021; Francoeur et al., 2020).

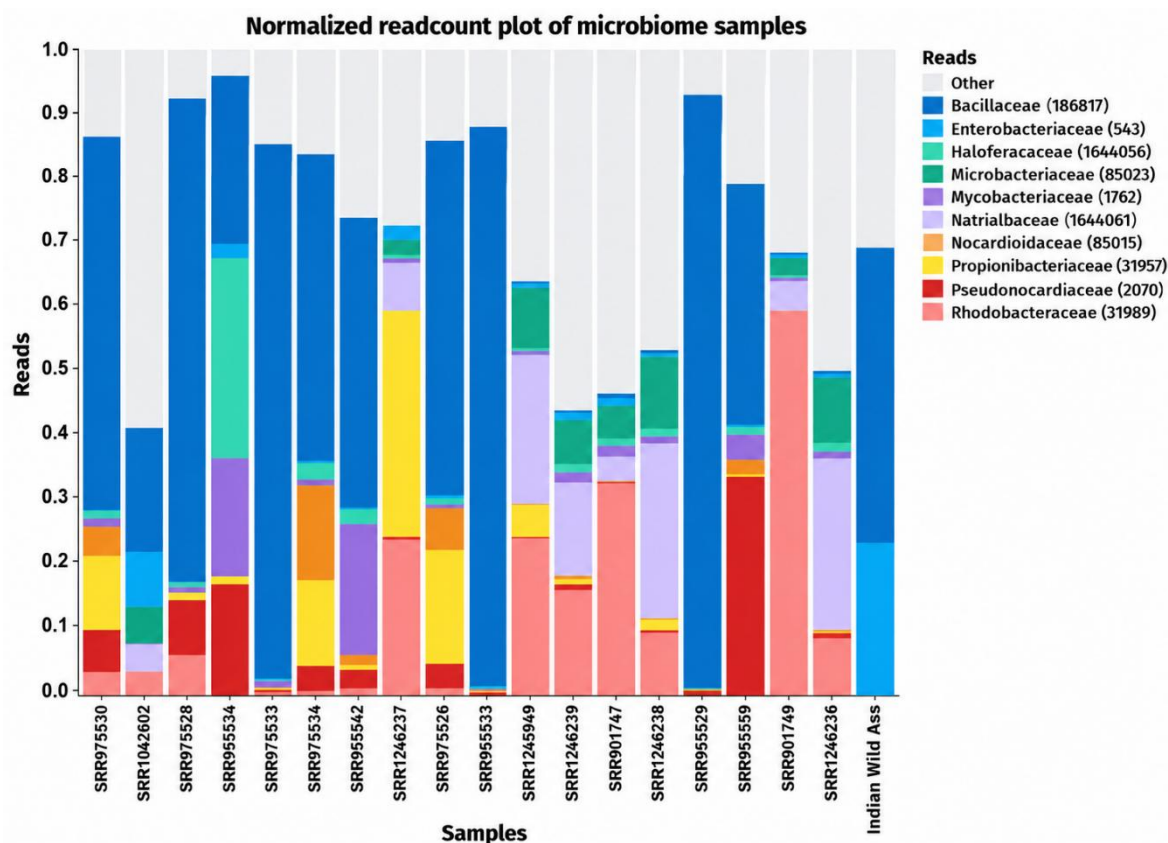


Figure 3 Comparative analysis of the Indian wild ass gut microbiome and saline soil microbiomes of the Kutch region at the family level. Each SRR number corresponds to a specific saline soil sample retrieved from the NCBI database. The figure highlights the taxonomic composition and relative abundance of bacterial families across gut and soil samples.

Patel et al. (2015) reported Alteromonadaceae as the central dominating family in saline soil microbiota from the wild ass sanctuary, Little Rann of Kutch. In contrast, the present study found a 0.02% abundance of this family in the gut microbiome of the Indian wild ass. The Alteromonadaceae family includes species adapted to various environmental conditions, including different salinities and

temperatures (Fotedar et al., 2020; Liao et al., 2017). Members of this family exhibit a range of metabolic capabilities, including the degradation of complex polysaccharides such as chitin and mannans. These organisms may enhance the overall digestive efficiency and nutrient absorption in herbivores by breaking down complex polysaccharides and other recalcitrant molecules present in their diet,

maintaining gut health and preventing dysbiosis (Ding et al., 2022; Sui et al., 2021).

At the genus level, *Bacillus* (5.41%) was reported most abundant from a total of 452 genera, followed by *Prevotella* (4.36%) and *Acinetobacter* (3.69%). The culturable bacterial isolates derived from the flora and soil of the Kutch saline region; notably, majority of these isolates were classified within the genus *Bacillus*. The soil from the Little Rann of Kutch (LRK) soil demonstrated a reduced organic content, whereas the diversity of the isolated bacteria exhibited adaptation to extreme environmental conditions. Most of these isolates were halophilic or haloalkaliphilic, tolerating temperatures up to 50°C. Many isolates secreted extracellular enzymes, indicating biotechnological potential, and exhibited plant growth-promoting properties (Bhatt & Singh, 2022; Rina et al., 2009). Twenty-four bacterial strains from the Indian wild ass excrement were classified as *Bacillus*, showing the ability to produce salt- and thermotolerant enzymes (Khunt et al., 2011). This is consistent with findings in other animals, where environmental microbes contributed significantly to the gut microbial community (Bornbusch et al., 2022; Pepper & Brooks, 2021; Qin et al., 2022; B. Sun et al., 2021; Tasnim et al., 2017; Zhou et al., 2018). The genus *Prevotella* was found in several equid species like Chinese ponies (Lv et al., 2023), horses (Stewart et al., 2019), Tibetan wild ass (Liu et al., 2022), donkeys (Y. Li et al., 2022), Przewalski's horses (Hu et al., 2021). It was remarkably prevalent in several ruminant species and played a crucial role in metabolic versatility, particularly in carbohydrate fermentation and propionate production, reducing methane emissions (Betancur-Murillo et al., 2023).

At the species level, most abundant was *Treponema brennaborensis* (3.41%) followed by *Auritiidibacter* sp. NML130574 (1.80%) and *Prevotella jejuni* (1.22%) from the Indian wild ass (*Equus hemionus khur*) gut microbiome. Other notably abundant species include *Prevotella dentalis*, *Croceibacter atlanticus*, *Fibrobacter succinogenes*, *Bacillus* sp. FJAT-22090, *Pseudoclostridium thermosuccinogenes*, *Escherichia coli*, *Akkermansia glycaniphila* (Figure 2). The species *Treponema brennaborensis* were reported in several ruminants (Yak, sheep, cattle, deer) and equids (Tibetan wild ass, Przewalski's gazelle) by CRAMdb (comprehensive database for composition and roles of microbiome in animals) database (Lei et al., 2023). *Croceibacter atlanticus*, possibly linked to the saline environment (Cho & Giovannoni, 2003), and *Fibrobacter succinogenes*, essential for cellulose degradation (Suen et al., 2011), were also identified. *Pseudoclostridium thermosuccinogenes* may contribute to energy metabolism through succinate production (Koendjibiharie et al., 2018). This diverse microbiome, comprising bacteria from various environments, indicates unique adaptations to the wild ass's diet and habitat (Costa & Weese, 2012). The presence of various lactobacillus species was found in the Indian wild ass gut microbiome (Table 1). The halotolerant lactobacilli have diverse applications in food

fermentation and agriculture due to their ability to thrive in hypersaline environments. The diverse *Lactobacillus* community contributes to a balanced gut ecosystem. This natural probiotic effect could help prevent dysbiosis and maintain overall gut health in wild asses. In Archaea, the phylum *Candidatus* *thermoplasmata* was found to have 0.07% total abundance.

Table 1 % Abundance of *Lactobacillus* species in Indian wild ass gut microbiome

<i>Lactobacillus</i> Species	% Abundance
<i>Lactobacillus delbrueckii</i> subsp. <i>bulgaricus</i>	0.002
<i>Companilactobacillus alimentarius</i> DSM 20249	0.002
<i>Fructilactobacillus sanfranciscensis</i> TMW 1.1304	0.002
<i>Lentilactobacillus buchneri</i> subsp. <i>silagei</i> CD034	0.005
<i>Lactocaseibacillus paracasei</i> subsp. <i>paracasei</i> 8700:2	0.01
<i>Lactobacillus kefirianofaciens</i> ZW3	0.012
<i>Ligilactobacillus ruminis</i> ATCC 27782	0.024
<i>Lactobacillus jensenii</i>	0.002
<i>Lactiplantibacillus plantarum</i>	0.002
<i>Lactobacillus acidophilus</i>	0.005
<i>Limosilactobacillus reuteri</i>	0.007
<i>Lactobacillus helveticus</i>	0.01
<i>Lactocaseibacillus rhamnosus</i>	0.012
<i>Ligilactobacillus murinus</i>	0.017
<i>Ligilactobacillus agilis</i>	0.022
<i>Ligilactobacillus acidipiscis</i>	0.044
<i>Ligilactobacillus salivarius</i>	0.046
<i>Limosilactobacillus fermentum</i>	0.061

Taxonomic to Phenotypic Characterisation

The gut microbiome of the Indian wild ass exhibits a diverse range of metabolic capabilities, habitat associations, and temperature adaptations. The significant presence of unknown phenotypes across all categories suggests that there is still much to learn about this microbial ecosystem. Understanding these unknowns could provide insights into novel metabolic pathways and microbial interactions crucial for the host's health and digestion.

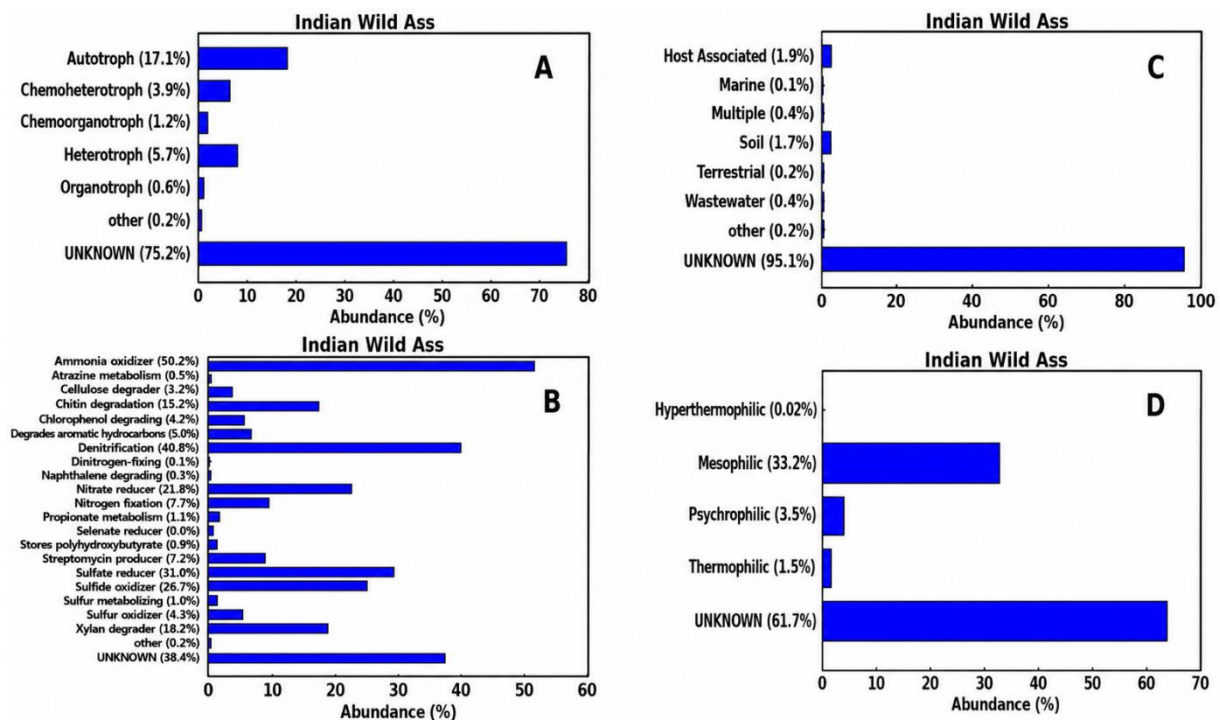


Figure 3 Phenotypic characteristics of the Indian wild ass Gut Microbiome. In the set of graphs, (A) illustrates the energy sources, (B) highlights the metabolic processes, (C) focuses on habitat parameters, and (D) represents temperature range adaptations.

The gut microbiome of the Indian wild ass demonstrates diverse metabolic, habitat, and temperature adaptations. Among the energy sources, autotrophs dominate (17.1%), followed by heterotrophs (5.7%) and chemoheterotrophs (3.9%), unlike typical mammalian gut microbiomes dominated by heterotrophs. Autotrophic microbes converting inorganic compounds into beneficial organics, aiding in pathogen defense and maintaining microbial balance (Yang *et al.*, 2022) (Figure 4A). In metabolic functions, ammonia oxidation is the most prominent (50.2%), followed by dehalogenation (40.8%) and sulfate reduction (31.0%). Sulfide oxidation and nitrite reduction are also significant, accounting for 26.7% and 21.8%, respectively. Other notable functions include xylan degradation (18.2%), chitin degradation (15.2%), nitrogen fixation (7.7%). Additionally, the microbiome is involved in the degradation of aromatic hydrocarbons (5.0%), chlorophenol (4.2%), and cellulose (3.2%), as well as sulfur oxidation (4.3%) and atrazine metabolism (0.5%). This diverse metabolic profile highlights the complex and multifaceted roles of the gut microbiome in the Indian wild ass (Figure 4B). Regarding habitat preferences, Host-associated environments dominate habitat preferences (1.9%), followed by soil habitats (1.7%), while marine (0.1%) and terrestrial habitats (0.2%) are minimally represented (Figure 4C). The temperature adaptations of the microbiome, ranging from psychrophilic to thermophilic, most microbiomes are mesophilic (33.2%), with psychrophiles (3.5%), thermophiles (1.5%), and minimal hyperthermophiles (0.02%) (Figure 4D). These temperature adaptations support host survival in variable environments (Kozioł *et al.*, 2023; X. Zhang *et al.*, 2022).

Metagenome PGPT Analysis

The metagenome analysis of plant growth-promoting traits (PGPT) indicates that the fecal bacterial diversity of the Indian wild ass possesses the capability to facilitate plant growth through mechanisms such as bioremediation, stimulation of plant immune responses, colonization of plant systems, stress regulation/biocontrol, mitigation of biotic stress, biodegradation of xenobiotics, detoxification of heavy metals, provision of bio-fertilization, utilization of plant-derived substrates for colonization, and solubilization of phosphates (Figure 5). From wild ass fecal very less microorganisms were cultured, while through our correlative observation of culturable and metagenomic bacterial diversity within the saline desert region of Kutch, we have ascertained that the fecal bacterial diversity of Indian wild ass significantly enhances both soil and plant health, as well as the endurance of the Indian wild ass in the saline desert of Kutch. An

increase in the population of wild asses could potentially result in broader dissemination of advantageous soil bacteria, improved soil fertility, and enhanced plant growth in areas exhibiting degradation, thereby augmenting the resilience of ecosystems to various environmental stresses.

Predictive Functional Profiling

The L1 profile pathway in the gut microbiome of the Indian wild ass shows a high abundance (69%) for metabolism, followed by genetic information processing pathways (12%). Organismal systems pathways account for 11% of the functional abundance, while cellular processes represent 7%. Environmental information processing pathways have the lowest abundance (1%). This distribution suggests that the Indian wild ass gut microbiome is primarily focused on nutrient processing, with significant involvement in host-microbe interactions and genetic activities (Figure 6). In the context of L2 profile pathways, multiple metabolic pathways show a higher proportion, including carbohydrate metabolism, amino acid metabolism, nucleotide metabolism, the metabolism of cofactors and vitamins, energy metabolism, lipid metabolism, xenobiotics biodegradation, and metabolism, as well as the metabolism of terpenoids and polyketides (Figure 7). The pathways delineated from L3 functional prediction encompass purine metabolism (2.92%) and pyrimidine metabolism (2.43%) derived from nucleotide metabolism, as well as glycine, serine, and threonine metabolism (1.82%), cysteine and methionine metabolism (1.55%), and alanine, aspartate, and glutamate metabolism (1.13%) originating from amino acid metabolism. A variety of pathways related to microbial interactions and their adaptive mechanisms were discovered, including carbon fixation pathways in prokaryotic organisms (1.59%), quorum sensing (1.36%), and resistance mechanisms against cationic antimicrobial peptides (CAMP) (1.03%). Additionally, pathways related to the cell cycle (1.46%), along with DNA replication and repair processes like homologous recombination (1.35%) and mismatch repair (1.17%), were also identified. In the context of energy metabolism, methane metabolism (1.31%) was documented. Additionally, the pathway analysis underscored the metabolism of organic compounds, which included butanoate (1.41%), propanoate (1.23%), and benzoate (0.84%), as well as pathways linked to the biosynthesis of vitamins such as B5, B7, and B9, detected in notable abundance (Figure 8).

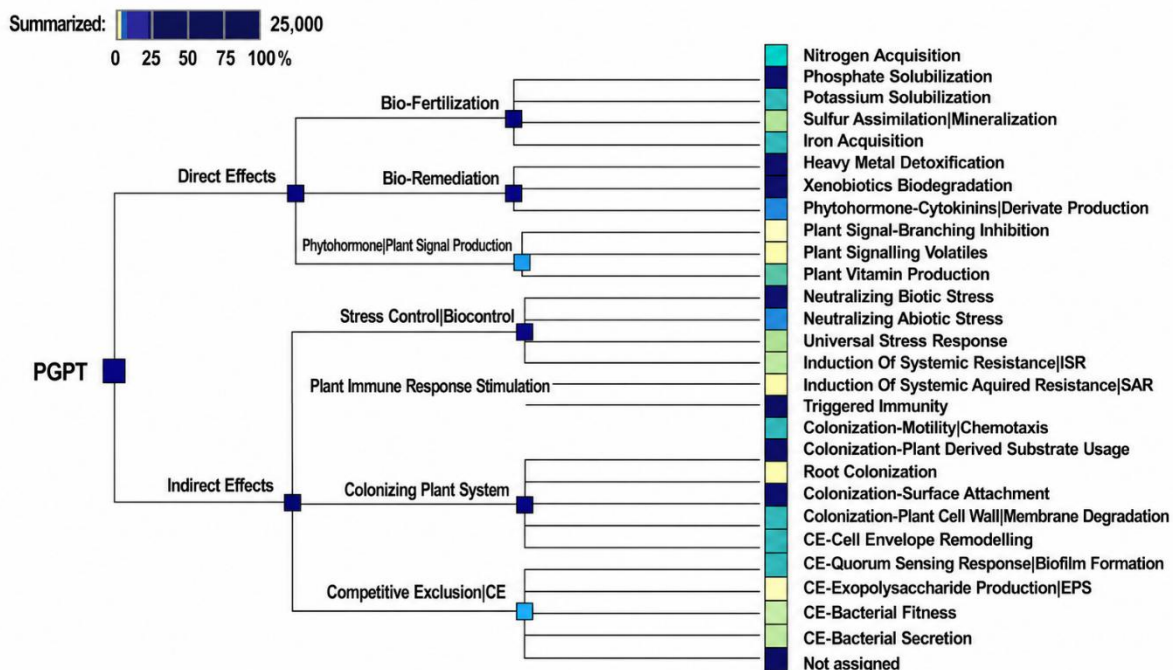


Figure 4 PGPT (Plant Growth-Promoting Trait) functional analysis of the Indian wild ass fecal metagenome. The heat map illustrates the functional categories associated with PGPTs under direct and indirect effects. Color intensity corresponds to the relative abundance of each functional trait, with darker shades indicating higher abundance based on summarized hit counts (0–25,000).

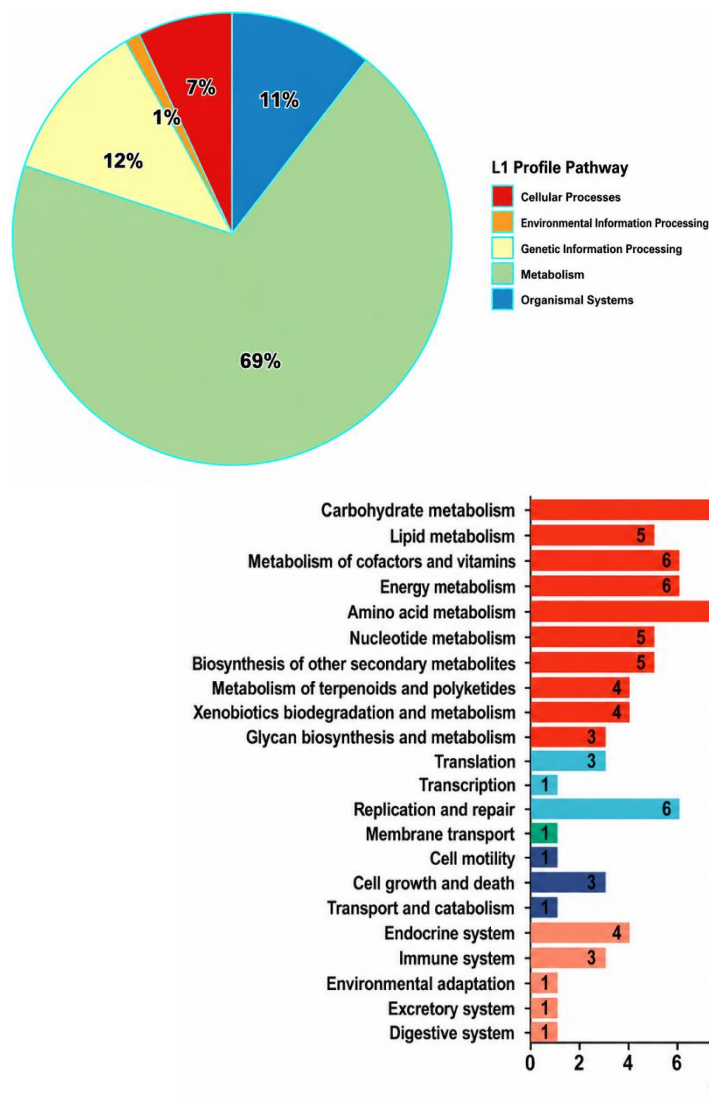


Figure 6 Relative abundance of Level 2 (L2) functional pathways in the Indian wild ass gut microbiome. Pathways are grouped according to their corresponding Level 1 functional categories

This adaptation allows the Indian wild ass to safely consume a variety of plant species, which is crucial in an environment where food sources may be scarce or unpredictable. Furthermore, the water-conserving metabolic pathways and the biosynthesis of unsaturated fatty acids indicate adaptations to the arid climate, enabling the animal to maintain physiological functions under water-limited conditions (El Shaer, 2010). Enhanced quorum sensing, bacterial chemotaxis, and diverse metabolic pathways suggest a highly responsive microbial community capable of rapidly adapting to changing food sources (Flint *et al.*, 2012; Frese *et al.*, 2015). This flexibility is essential given the potential for seasonal variations in food availability in the Little Rann of Kutch ecosystem.

CONCLUSION

This metagenomic study provides a comprehensive characterization of the gut microbiome of the Indian wild ass (*Equus hemionus khur*) revealing unique compositional and functional features that reflect adaptations to its saline desert habitat. The microbiome is highly diverse, with major phyla such as Firmicutes, Bacteroidetes and proteobacteria, with notable abundance of halophilic and halotolerant species. Investigation of the predictive functional role of these microorganisms revealed various elements of their biochemistry; increased lipid metabolism and biodegradation of xenobiotics show the complex biochemical role of bacteria this specialized environment. From an environmental microbiology perspective, the presence of autotrophic microbes and varied temperature adaptations demonstrates the resilience of microbial communities in harsh conditions. The research also gives an outlook of the host-microbe association, suggesting how the gut microbiota might influence the health and adaptation of the endangered wild ass to its saline habitat. Notably, the identification of

Figure 5 functional abundance percentages of various biological processes (L1-level 1). The processes are categorized into Metabolism, Genetic Information Processing, Environmental Information Processing, Cellular Processes, and Organismal Systems.

The diverse diet consisting of various grasses, sedges, and legumes, is reflected in the metabolic diversity of the microbiome. The high proportion of pathways related to carbohydrate, amino acid, and fatty acid metabolism, butanoate metabolism, pentose phosphate pathway, and glycerophospholipid metabolism, starch and sucrose metabolism, biosynthesis of vitamins indicates an efficient system for extracting nutrients from fibrous plant material while maintaining cellular integrity under osmotic stress (Julliaud & Grimm, 2016; Rangan *et al.*, 2014). Enhanced detoxification pathways, benzoate degradation, glutathione metabolism, and increased peroxisome activity suggest an evolved ability to handle the secondary metabolites present in plants (Kohl *et al.*, 2014)

microorganisms with plant growth-promoting traits opens avenues for studying plant associated microbiota in extreme habitats. The high-salt-adapted microbes could be considered for new applications in bioremediation strategies, salt-tolerant enzymes, and agricultural uses for saline soils. The research presents opportunities for microbial systems biology studies, potentially leading to new understandings of microbial community dynamics and interactions. Furthermore, the diverse microbial profiles observed could inform future investigations into novel antimicrobials and biofilm formation in hypersaline conditions. By providing a comprehensive analysis of a previously unexplored microbiome, this study not only advances our understanding of microbial ecology but also lays the groundwork for future research in various aspects of microbial sciences, from genetics and metabolism to environmental adaptations and host-microbe relationships.

Ethical Justification: Fecal samples from the Indian wild asses were collected in open, non-restricted areas with the assistance of the local residential community. The process was entirely non-invasive, as only fresh, leftover fecal material was gathered, ensuring that no harm or disturbance occurred to the animals or their habitat. Since the method did not involve any direct interaction or interference with the wildlife, no formal ethical permission was required for this study.

Declaration of Competing Interest: The authors declare that they have no competing interests.

Data availability: The sequencing raw reads of this study have been deposited in the NCBI SRA under the Bio Project: PRJNA1170020.
<https://dataview.ncbi.nlm.nih.gov/object/PRJNA1170020?reviewer=h57hnl0k56ms3dgdub1oprgkj>

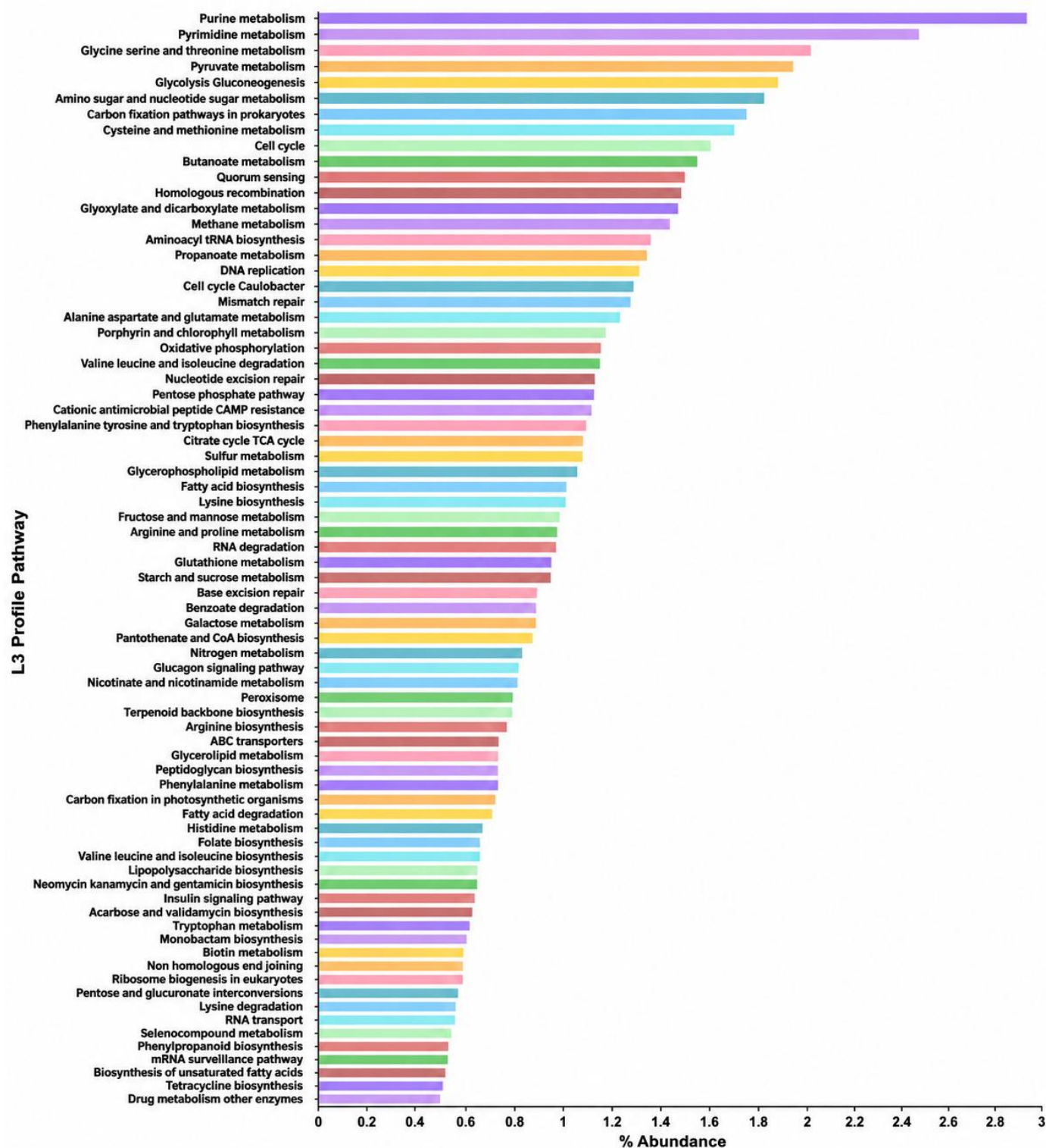


Figure 7 Level 3 (L3) profile pathway analysis of the Indian wild ass gut microbiome showing the relative abundance (% abundance) of various metabolic pathways. Different colors are used to visually differentiate each pathway and do not indicate functional groupings or classifications.

REFERENCES

- Anteneh, Y. S., & Franco, C. M. M. (2017). Symbiosis and pathogenicity of actinobacteria. In *Biology and Biotechnology of Actinobacteria*. https://doi.org/10.1007/978-3-319-60339-1_9
- Antwis, R. E., Lea, J. M. D., Unwin, B., & Shultz, S. (2018). Gut microbiome composition is associated with spatial structuring and social interactions in semi-feral Welsh Mountain ponies. *Microbiome*, 6(1). <https://doi.org/10.1186/s40168-018-0593-2>
- Andrews, S. (2017). FastQC: a quality control tool for high throughput sequence data. 2010.
- Arndt, D., Xia, J., Liu, Y., Zhou, Y., Guo, A. C., Cruz, J. A., Sinelnikov, I., Budwill, K., Nesbø, C. L., & Wishart, D. S. (2012). METAGENassist: A comprehensive web server for comparative metagenomics. *Nucleic Acids Research*, 40(W1). <https://doi.org/10.1093/nar/gks497>
- Baldelli, V., Scaldaferrì, F., Putignani, L., & Del Chierico, F. (2021). The role of enterobacteriaceae in gut microbiota dysbiosis in inflammatory bowel diseases. In *Microorganisms* (Vol. 9, Issue 4). <https://doi.org/10.3390/microorganisms9040697>
- Bao, W., Yu, J., He, Y., Liu, M., & Yang, X. (2022). The diversity analysis and gene function prediction of intestinal bacteria in three equine species. *Frontiers in Microbiology*, 13. <https://doi.org/10.3389/fmicb.2022.973828>
- Barathan, M., Ng, S. L., Lokanathan, Y., Ng, M. H., & Law, J. X. (2024). The Profound Influence of Gut Microbiome and Extracellular Vesicles on Animal Health and Disease. In *International Journal of Molecular Sciences* (Vol. 25, Issue 7). Multidisciplinary Digital Publishing Institute (MDPI). <https://doi.org/10.3390/ijms25074024>

- Barman, B. B., Shah, N., Prasad, A., Sanyal, A., Chavda, V., & Qureshi, Q. (2022). Monitoring Spatial and Seasonal Abundance of Indian Wild Ass (*Equus hemionus khur*) in Little Rann of Kutch Landscape, Western India. *Journal of Ecophysiology and Occupational Health*, 21(4). <https://doi.org/10.18311/jecoh/2021/29033>
- Betancur-Murillo, C. L., Aguilar-Marin, S. B., & Jovel, J. (2023). Prevotella: A Key Player in Ruminant Metabolism. In *Microorganisms* (Vol. 11, Issue 1). <https://doi.org/10.3390/microorganisms11010001>
- Bhatt, H. B., & Singh, S. P. (2022). Diversity of cultivable bacteria in a saline desert of Little Rann of Kutch, India: a phylogenetic perspective. *Frontiers in Marine Science*, 9, 769043.
- Breitwieser, F. P., & Salzberg, S. L. (2020). Pavian: Interactive analysis of metagenomics data for microbiome studies and pathogen identification. *Bioinformatics*, 36(4). <https://doi.org/10.1093/bioinformatics/btz715>
- Chaucheyras-Durand, F., Sacy, A., Karges, K., & Apper, E. (2022). Gastro-Intestinal Microbiota in Equines and Its Role in Health and Disease: The Black Box Opens. In *Microorganisms* (Vol. 10, Issue 12). <https://doi.org/10.3390/microorganisms10122517>
- Cho, J. C., & Giovannoni, S. J. (2003). *Croceibacter atlanticus* gen. nov., sp. nov., a novel marine bacterium in the family Flavobacteriaceae. *Systematic and Applied Microbiology*, 26(1). <https://doi.org/10.1078/072320203322337344>
- Costa, M. C., & Weese, J. S. (2012). The equine intestinal microbiome. In *Animal health research reviews / Conference of Research Workers in Animal Diseases* (Vol. 13, Issue 1). <https://doi.org/10.1017/S1466252312000035>
- Dapa, T., & Xavier, K. B. (2024). Effect of diet on the evolution of gut commensal bacteria. In *Gut Microbes* (Vol. 16, Issue 1). Taylor and Francis Ltd. <https://doi.org/10.1080/19490976.2024.2369337>
- Dearing, M. D., & Kohl, K. D. (2017). Beyond fermentation: Other important services provided to endothermic herbivores by their gut microbiota. *Integrative and Comparative Biology*, 57(4). <https://doi.org/10.1093/icb/ixc020>
- Ding, R., Xie, H., Han, Z., & Yang, J. (2022). Production and Functional Characterization of a Novel Mannanase from Alteromonadaceae Bacterium Bs31. *Protein & Peptide Letters*, 29(8). <https://doi.org/10.2174/0929866529666220615161603>
- Edwards, J. E., Shetty, S. A., van den Berg, P., Burden, F., van Doorn, D. A., Pellikaan, W. F., Dijkstra, J., & Smidt, H. (2020). Multi-kingdom characterization of the core equine fecal microbiota based on multiple equine (sub)species. *Animal Microbiome*, 2(1). <https://doi.org/10.1186/s42523-020-0023-1>
- El Shaer, H. M. (2010). Halophytes and salt-tolerant plants as potential forage for ruminants in the Near East region. In *Small Ruminant Research* (Vol. 91, Issue 1). <https://doi.org/10.1016/j.smallrumres.2010.01.010>
- Flint, H. J., Scott, K. P., Duncan, S. H., Louis, P., & Forano, E. (2012). Microbial degradation of complex carbohydrates in the gut. In *Gut Microbes* (Vol. 3, Issue 4). <https://doi.org/10.4161/gmic.19897>
- Fotadar, R., Caldwell, M. E., Sankaranarayanan, K., Al-Zeyara, A., Al-Malki, A., Kaul, R., Al Marri, M., Al-Shamari, H. S., & Lawson, P. A. (2020). *Ningiella ruwaisensis* gen. Nov., sp. nov., a member of the family alteromonadaceae isolated from marine water of the Arabian Gulf. *International Journal of Systematic and Evolutionary Microbiology*, 70(7). <https://doi.org/10.1099/ijsem.0.004256>
- Francoeur, C. B., Khadempour, L., Moreira-Soto, R. D., Gotting, K., Book, A. J., Pinto-Tomás, A. A., Keefover-Ring, K., & Currie, C. R. (2020). Bacteria contribute to plant secondary compound degradation in a generalist herbivore system. *MBio*, 11(5). <https://doi.org/10.1128/mbio.02146-20>
- Frese, S. A., Parker, K., Calvert, C. C., & Mills, D. A. (2015). Diet shapes the gut microbiome of pigs during nursing and weaning. *Microbiome*, 3(1). <https://doi.org/10.1186/s40168-015-0091-8>
- Gao, H., Chi, X., Li, G., Qin, W., Song, P., Jiang, F., Liu, D., Zhang, J., Zhou, X., Li, S., & Zhang, T. (2020). Gut microbial diversity and stabilizing functions enhance the plateau adaptability of Tibetan wild ass (*Equus Kiang*). *MicrobiologyOpen*, 9(6). <https://doi.org/10.1002/mbo3.1025>
- Gao, Y., Li, D., & Liu, Y. X. (2023). Microbiome research outlook: past, present, and future. *Protein & Cell*, 14(10), 709-712.
- Gao, H., Jiang, F., Zhang, J., Chi, X., Song, P., Bin, L. I., Cai, Z., & Zhang, T. (2023). Effects of ex situ conservation on diversity and function of the gut microbiota of the Tibetan wild ass (*Equus Kiang*). *Integrative Zoology*, 18(6). <https://doi.org/10.1111/1749-4877.12726>
- Gee, E. P. (1963). The indian wild ass. *Oryx*, 7(1), 9-21. <https://doi.org/10.1017/S0030605300002192>
- Gosalbes, M. J., Durbán, A., Pignatelli, M., Abellán, J. J., Jiménez-Hernández, N., Pérez-Cobas, A. E., Latorre, A., & Moya, A. (2011). Metatranscriptomic approach to analyze the functional human gut microbiota. *PLoS ONE*, 6(3). <https://doi.org/10.1371/journal.pone.0017447>
- Hammer, Ø., Harper, D. A. T., & Ryan, P. D. (2001). Past: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica*, 4(1).
- Hu, D., Yang, J., Qi, Y., Li, B., Li, K., & Mok, K. M. (2021). Metagenomic Analysis of Fecal Archaea, Bacteria, Eukaryota, and Virus in Przewalski's Horses Following Anthelmintic Treatment. *Frontiers in Veterinary Science*, 8. <https://doi.org/10.3389/fvets.2021.708512>
- Huson, D. H., Auch, A. F., Qi, J., & Schuster, S. C. (2007). MEGAN analysis of metagenomic data. *Genome Research*, 17(3). <https://doi.org/10.1101/gr.5969107>
- International Science Community Association. (n.d.).
- Julliand, V., & Grimm, P. (2016). Horse species symposium: The microbiome of the horse hindgut: History and current knowledge. In *Journal of Animal Science* (Vol. 94, Issue 6). <https://doi.org/10.2527/jas.2015-0198>
- Khair, D., Atkulwar, A., Farah, S., & Baig, M. (2017). Mitochondrial DNA analyses revealed low genetic diversity in the endangered Indian wild ass *Equus hemionus khur*. *Mitochondrial DNA Part A: DNA Mapping, Sequencing, and Analysis*, 28(5). <https://doi.org/10.3109/24701394.2016.1174221>
- Khunt, M., & Pandhi, N. (2011). Moderate halophilic bacterial community in excreta of wild ass (*Equus hemionus khur*). *International journal of Bioscience*, 1(5), 31-37.
- Kittelmann, S., Seedorf, H., Walters, W. A., Clemente, J. C., Knight, R., Gordon, J. I., & Janssen, P. H. (2013). Simultaneous Amplicon Sequencing to Explore Co-Occurrence Patterns of Bacterial, Archaeal and Eukaryotic Microorganisms in Rumen Microbial Communities. *PLoS ONE*, 8(2). <https://doi.org/10.1371/journal.pone.0047879>
- Koendjibiharie, J. G., Wiersma, K., & van Kranenburg, R. (2018). Investigating the central metabolism of *Clostridium thermosuccinogenes*. *Applied and Environmental Microbiology*, 84(13). <https://doi.org/10.1128/AEM.00363-18>
- Kohl, K. D., Weiss, R. B., Cox, J., Dale, C., & Dearing, M. D. (2014). Gut microbes of mammalian herbivores facilitate intake of plant toxins. *Ecology Letters*, 17(10). <https://doi.org/10.1111/ele.12329>
- Kozioł, A., Odriozola, I., Leonard, A., Eisenhofer, R., José, C. S., Aizpurua, O., & Alberdi, A. (2023). Mammals show distinct functional gut microbiome dynamics to identical series of environmental stressors. *MBio*, 14(5). <https://doi.org/10.1128/mbio.01606-23>
- La Rosa, S. L., Leth, M. L., Michalak, L., Hansen, M. E., Pudlo, N. A., Glowacki, R., Pereira, G., Workman, C. T., Arntzen, M., Pope, P. B., Martens, E. C., Hachem, M. A., & Westereng, B. (2019). The human gut Firmicute *Roseburia intestinalis* is a primary degrader of dietary β -mannans. *Nature Communications*, 10(1). <https://doi.org/10.1038/s41467-019-08812-y>
- Latha, S., & Dhanasekaran, D. (2013). Antibacterial and extracellular enzyme activities of gut actinobacteria isolated from Gallus domesticus and Capra hircus. *Journal of Chemical and Pharmaceutical Research*, 5(11).
- Lee, J. W., Cowley, E. S., Wolf, P. G., Dodson, H. L., Murai, T., Caicedo, K. Y. O., Ly, L. K., Sun, F., Takei, H., Nittono, H., Daniel, S. L., Cann, I., Gaskins, H. R., Anantharaman, K., Alves, J. M. P., & Ridlon, J. M. (2022). Formation of secondary allo-bile acids by novel enzymes from gut Firmicutes. *Gut Microbes*, 14(1). <https://doi.org/10.1080/19490976.2022.2132903>
- Lei, B., Xu, Y., Lei, Y., Li, C., Zhou, P., Wang, L., Yang, Q., Li, X., Li, F., Liu, C., Cui, C., Chen, T., Ni, W., & Hu, S. (2023). CRAMdb: a comprehensive database for composition and roles of microbiome in animals. *Nucleic Acids Research*, 51(1 D). <https://doi.org/10.1093/nar/gkac973>
- Leth, M. L., Pichler, M. J., & Hachem, M. A. (2023). Butyrate-producing colonic clostridia: picky glycan utilization specialists. In *Essays in Biochemistry* (Vol. 67, Issue 3). <https://doi.org/10.1042/EBC20220125>
- Li, Y., Ma, Q., Shi, X., Liu, G., & Wang, C. (2022). Integrated multi-omics reveals novel microbe-host lipid metabolism and immune interactions in the donkey hindgut. *Frontiers in Immunology*, 13. <https://doi.org/10.3389/fimmu.2022.1003247>
- Liao, H., Li, Y., Guo, X., Lin, X., Lai, Q., Xu, H., Zheng, T., & Tian, Y. (2017). *Mangrovitalea sediminis* gen. Nov., sp. nov., a member of the family alteromonadaceae isolated from mangrove sediment. *International Journal of Systematic and Evolutionary Microbiology*, 67(12). <https://doi.org/10.1099/ijsem.0.002433>
- Liu, H., Han, X., Zhao, N., Hu, L., Wang, X., Luo, C., Chen, Y., Zhao, X., & Xu, S. (2022). The Gut Microbiota Determines the High-Altitude Adaptability of Tibetan Wild Asses (*Equus Kiang*) in Qinghai-Tibet Plateau. *Frontiers in Microbiology*, 13. <https://doi.org/10.3389/fmicb.2022.949002>
- Lv, S., Zhang, Y., Zhang, Z., Meng, S., Pu, Y., Liu, X., Liu, L., Ma, Y., Liu, W., & Jiang, L. (2023). Diversity of the fecal microbiota in Chinese ponies. *Frontiers in Veterinary Science*, 10. <https://doi.org/10.3389/fvets.2023.1102186>
- Nagpal, S., Haque, M. M., & Mande, S. S. (2016). Vikodak - A modular framework for inferring functional potential of microbial communities from 16S metagenomic datasets. *PLoS ONE*, 11(2). <https://doi.org/10.1371/journal.pone.0148347>
- Patz, S., Rauh, M., Gautam, A., & Huson, D. H. (2024). mgPGPT: Metagenomic analysis of plant growth-promoting traits. *bioRxiv*, 2024-02.
- Paul, L. J., Ericsson, A. C., Andrews, F. M., McAdams, Z., Keowen, M. L., St Blanc, M. P., & Banse, H. E. (2022). Dietary and management factors influence the equine gastric microbiome. *Journal of the American Veterinary Medical Association*, 260. <https://doi.org/10.2460/javma.22.07.0277>
- Pokharia, A. K., Basumatary, S. K., Thakur, B., Tripathi, S., McDonald, H. G., Tripathi, D., Tiwari, P., Van Asperen, E., Spate, M., Chauhan, G., Thakkar, M. G., Srivastava, A., & Agarwal, S. (2022). Multiproxy analysis on Indian wild ass (*Equus hemionus khur*) dung from Little Rann of Western India and its implications for the palaeoecology and archaeology of arid regions. *Review of Palaeobotany and Palynology*, 304. <https://doi.org/10.1016/j.revpalbo.2022.104700>
- Prasad, S. N., Goyal, S. P., Roy, P. S., & Singh, S. (1994). Changes in wild ass (*Equus hemionus khur*) habitat conditions in Little Rann of Kutch, Gujarat from a

- remote sensing perspective. *International Journal of Remote Sensing*, 15(16). <https://doi.org/10.1080/01431169408954318>
- Rangan, P., Subramani, R., Kumar, R., Singh, A. K., & Singh, R. (2014). Recent advances in polyamine metabolism and abiotic stress tolerance. In *BioMed Research International* (Vol. 2014). <https://doi.org/10.1155/2014/239621>
- Raval, P. P. (1988). The Indian wild ass —wild and captive populations. *Oryx*, 22(2). <https://doi.org/10.1017/S003060530002754X>
- Rina, K., Hiral, P., & Payal, P. (2009). Study on Microbial Diversity of Wild Ass. *IUP Journal of Life Sciences*, 3.
- Singh, H. S. (2000). Status of Indian Wild Ass (*Equus hemionus khur*) in the Little Rann of Kutch. *Zoos' Print Journal*, 15(5). <https://doi.org/10.11609/jott.zpj.15.5.253-6>
- Singh, H. S., & Soni, V. C. (1999). Status of wildlife in wild ass sanctuary (Little Rann of Kutch, Gujarat State, India). *Indian Forester*, 125(10).
- Stewart, H. L., Southwood, L. L., Indugu, N., Vecchiarelli, B., Engiles, J. B., & Pitta, D. (2019). Differences in the equine faecal microbiota between horses presenting to a tertiary referral hospital for colic compared with an elective surgical procedure. *Equine Veterinary Journal*, 51(3). <https://doi.org/10.1111/evj.13010>
- Suen, G., Weimer, P. J., Stevenson, D. M., Aylward, F. O., Boyum, J., Deneke, J., Drinkwater, C., Ivanova, N. N., Mikhailova, N., Chertkov, O., Goodwin, L. A., Currie, C. R., Mead, D., & Brumm, P. J. (2011). The complete genome sequence of fibrobacter succinogenes s85 reveals a cellulolytic and metabolic specialist. *PLoS ONE*, 6(4). <https://doi.org/10.1371/journal.pone.0018814>
- Sui, X., He, X. yan, Liu, N. hua, Dang, Y. ru, Cha, Q. qian, Sun, M. ling, Li, C. yang, Fu, H. hui, Song, X. yan, Qin, Q. long, Chen, X. lan, Zhang, Y. zhong, Su, H. nan, & Zhang, X. ying. (2021). *Marinifaba aquimaris* gen. nov., sp. nov., a novel chitin-degrading gammaproteobacterium in the family Alteromonadaceae isolated from seawater of the Mariana Trench. *Antonie van Leeuwenhoek, International Journal of General and Molecular Microbiology*, 114(7). <https://doi.org/10.1007/s10482-021-01568-w>
- Sun, Y., Zhang, S., Nie, Q., He, H., Tan, H., Geng, F., Ji, H., Hu, J., & Nie, S. (2023). Gut firmicutes: Relationship with dietary fiber and role in host homeostasis. In *Critical Reviews in Food Science and Nutrition* (Vol. 63, Issue 33). <https://doi.org/10.1080/10408398.2022.2098249>
- Tang, L., Gao, Y., Yan, L., Jia, H., Chu, H., Ma, X., He, L., Wang, X., Li, K., Hu, D., & Zhang, D. (2022). Comparative Analysis of Microbiome Metagenomics in Reintroduced Wild Horses and Resident Asiatic Wild Asses in the Gobi Desert Steppe. *Microorganisms*, 10(6). <https://doi.org/10.3390/microorganisms10061166>
- Wood, D. E., & Salzberg, S. L. (2014). Kraken: Ultrafast metagenomic sequence classification using exact alignments. *Genome Biology*, 15(3). <https://doi.org/10.1186/gb-2014-15-3-r46>
- Yang, Y., Sun, J., Chen, C., Zhou, Y., Van Dover, C. L., Wang, C., ... & Qian, P. Y. (2022). Metagenomic and metatranscriptomic analyses reveal minor-yet-crucial roles of gut microbiome in deep-sea hydrothermal vent snail. *Animal Microbiome*, 4, 1-17. <https://doi.org/10.1186/s42523-021-00150-z>
- Zhang, J., Kobert, K., Flouri, T., & Stamatakis, A. (2014). PEAR: A fast and accurate Illumina Paired-End reAd mergeR. *Bioinformatics*, 30(5). <https://doi.org/10.1093/bioinformatics/btt593>
- Zhang, X., Cui, K., Wen, X., Li, L., Yu, X., Li, B., Lin, H., He, H., & Wang, F. (2022). The Association between Gut Microbiome Diversity and Composition and Heat Tolerance in Cattle. *Microorganisms*, 10(8). <https://doi.org/10.3390/microorganisms10081672>
- Zhu, Y., Wang, X., Deng, L., Chen, S., Zhu, C., & Li, J. (2021). Effects of pasture grass, silage, and hay diet on equine fecal microbiota. *Animals*, 11(5). <https://doi.org/10.3390/ani11051330>