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Captive environments reshape the compositions of carbohydrate active enzymes and virulence factors in wolf gut microbiome

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Abstract

Species in the family Canidae occupy different spatial ecological niches, and some (e.g., wolf) can be kept in zoos. The gut microbiome may differ among various wild and captive canids. Therefore, we compared the gut microbiomes of wild canids (wolf, red fox, and corsac fox) in the Hulun Lake area, captive wolves, and domestic dogs in different regions using metagenomic data. A random forest analysis revealed significant enrichment for bacterial species producing short-chain fatty acids and the thermogenesis pathway (ko04714) in the gut microbiome of wild wolf, potentially providing sufficient energy for adaptation to a wide range of spatial ecological niches. The significantly enriched bacterial species and functional pathways in the gut microbiome of corsac foxes were related to physiological stability and adaptation to arid environments. Alpha diversity of carbohydrate-active enzymes in the gut microbiome was higher in the red fox than in the corsac fox and wild wolf, which may be related to the abundance of plant seeds (containing carbohydrates) in their diets (red foxes inhabit seed-rich willow bosk habitats). However, the influence of host genetic factors cannot be excluded, and further experimental studies are needed to verify the study results. In addition, captive environments drove similarity in carbohydrate-active enzymes (CAZymes) and virulence factors (VFs) in the gut microbiomes of captive wolf and domestic dog, and increased the diversity of CAZymes and VFs in the gut microbiome of captive wolf. Increased VFs diversity may increase the pathogenic potential of the gut microbiome in captive wolves. Therefore, it is necessary to continue monitoring the health status of captive wolves and develop appropriate management strategies.

Keywords Gut microbiome, Family canidae, Captive environmental, Ecological niches

Introduction

The gut microbiome is a complex ecosystem including bacteria, fungi, viruses, and archaea [1–3]. The composition of the gut microbiota influences host metabolism and thereby contributes to the response to challenges [3–6]. For example, in male Brandt's voles (*Lasiopodomys brandtii*), adaptation to low temperatures is related to increases the abundance of taxa producing short-chain fatty acids (SCFAs), which can increase thermogenesis by activating cAMP–PKA–pCREB signaling [6]. In pigs, host gene expression and energy metabolism are altered to cope with cold environments via alterations in the

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abundance of gut microbial taxa and metabolites [7]. Imbalances in the gut microbiome can lead to an increase in pathogenic bacteria, thereby enhancing the abundance of virulence factors (VFs) and affecting animal health [8]. Similarly, an increase in beneficial taxa in the microbiota can reduce the abundance of pathogenic bacteria and increase production of antibiotics and SCFAs, thereby enhancing host immunity and gut homeostasis [9, 10]. For example, *Clostridium scindens* secretes tryptophan-derived antibiotics (1-acetyl-β-carboline), which effectively inhibit the growth of pathogenic bacteria and maintain gut homeostasis [11].

Wild canids occupy different spatial ecological niches due to differences in body size and behavior [12–15]. Spatial ecological niches and behavioral patterns can lead to variation in the dietary ecological niche of canids [13, 16]. In the eastern region of Inner Mongolia, wild wolves (*Canis lupus*) have the widest spatial distribution among canids, followed by red foxes, while corsac foxes (*Vulpes corsac*) have the narrowest ecological niche and predominantly inhabit arid regions [17]. Microbiome sequencing is frequently used to investigate the mechanisms underlying animal ecological niche differentiation [18, 19]. For example, each folivorous lemur species (*Propithecus diadema*, *Indri indri*, *Avahi laniger*, and *Lepilemur mustelinus*) possesses a distinct set of gut microbial taxa for survival in different dietary niches (i.e., under different nutritional compositions) in the same environment [4]. The gut microbiome of red and corsac foxes have different abilities to adapt to different ecological niches [18]. In addition, as there are zoos with captive wolves throughout China, many studies have focused on the impact of environmental factors on the gut microbiome of wolf. *Succinivibrio*, *Turicibacter*, and *Prevotellaceae_Ga6A1_group* can protect captive wolves against pressures associated with human interference [20]. The gut microbiome of captive wolves was more similar to that of domestic dogs than to that of wild wolves [21]. However, the influence of captive environments on the gut microbiome of wolf has not been determined.

In this study, a comparative analysis of metagenomic data from wild canids (from eastern Inner Mongolia), captive wolves (from multiple regions), and domestic dogs (from multiple regions) was performed. We screened for potential microbial taxa and functional pathways related to wild canid adaptation to different ecological niches and evaluated the impact of captive environments on the wolf gut microbiome.

Materials and methods

Sample collection

Publicly available data were obtained for samples meeting the following standards. (1) Fecal samples were collected from wild Canidae species, frozen immediately after discharge, and encased in an ice shell to minimize contamination by the natural environment. (2) Samples were obtained from healthy captive Canidae species, placed in sterile tubes, and stored at -80 °C until sequencing. (3) Fresh feces were subjected to 150 bp paired-end sequencing (Illumina, shotgun metagenomics). In total, metagenomic data for 36 samples were downloaded from the Sequence Read Archive (SRA) database of the National Center for Biotechnology Information (NCBI), including data for three wild wolves, three wild red foxes, three wild corsac foxes, eight captive wolves, and seven domestic dogs. Chen et al. used GPS collars to track wild wolves and collected three fecal samples from wild wolves [21]. Wang et al. collected feces from three wild red foxes and three corsac foxes using the snow-fresh tracking method [18]. These feces of wild canids were from different individuals. Samples from captive wolves (Captive wolf 1–4 and 5–8 were the same four individuals) and domestic dog (metagenomic data for seven individuals) were collected from the same captive environment [21, 22]. To assess the effect of the captive environment on the gut microbiome of wolf, metagenomic data from wolves and domestic dogs in other captive environments were included, including five metagenomic data sets for captive wolves from Shenyang Forest Zoological Garden and Changchun Plant and Animal Park (Captive wolf II), and seven metagenomic data sets for domestic dogs from southern China (Domestic dog II) [23, 24]. These 12 metagenomes also followed the standards listed above. The sampling dates for 36 samples were from 2016 to 2022. Information on all metagenomic data is shown in Table 1.

Sequence processing and statistical analyses

We used fastp software (V 0.12.1) to eliminate adapters and low-quality bases from the raw data [25]. Similarly to whole–genome data of wolf [26], domestic dog [27], and red and corsac fox [28, 29], we employed Bowtie2 software (V 2.5.1) to filter host reads, and obtained the effective reads [30]. Based on the effective reads, EasyAmplicon (V 1.18.1) software was used for the standardization of sequencing depths [31]. Species annotation (from kingdom to species) of the gut microbiome was performed using Kraken2 (V 2.1.2) and Bracken (V 2.9; readLen = 150, retaining only taxa observed in 20% of all samples) [32]. The effective reads were assembled using Megahit (V 1.2.9; parameters, meta-sensitive), and contigs ≥ 500 bp were screened for subsequent analyses [33]. Prodigal software (V 2.6.3) was used to identify open reading frames (ORFs) within contigs [34]. Based on greater than or equal to 95% similarity threshold (no gaps) and over 90% coverage, an integrated non-redundant gene catalog was constructed using CD-HIT (V 4.8.1) [35]. Salmon (V 1.8) was used to quantify the

Table 1 The information of metagenomic data (All data were downloaded from the SRA database of NCBI)

Sample ID	Group	Environment	SRA number	Gender	Age	Location
Wild wolf 1	Wild wolf	Wild	SRR21169627	unknown	unknown	Hulun Lake area
Wild wolf 2	Wild wolf	Wild	SRR21169629	unknown	unknown	Hulun Lake area
Wild wolf 3	Wild wolf	Wild	SRR21169630	unknown	unknown	Hulun Lake area
Captive wolf 1	Captive wolf I	Captive	SRR7221654	male	adult	Hulun Lake area
Captive wolf 2	Captive wolf I	Captive	SRR7221655	male	adult	Hulun Lake area
Captive wolf 3	Captive wolf I	Captive	SRR7221653	female	adult	Hulun Lake area
Captive wolf 4	Captive wolf I	Captive	SRR7221652	female	adult	Hulun Lake area
Captive wolf 5	Captive wolf I	Captive	SRR21169623	female	adult	Hulun Lake area
Captive wolf 6	Captive wolf I	Captive	SRR21169624	female	adult	Hulun Lake area
Captive wolf 7	Captive wolf I	Captive	SRR21169625	male	adult	Hulun Lake area
Captive wolf 8	Captive wolf I	Captive	SRR21169626	male	adult	Hulun Lake area
Captive wolf 9	Captive wolf II	Captive	SRR13765889	unknown	adult	Shenyang Forest Zoological Garden
Captive wolf 10	Captive wolf II	Captive	SRR13765888	unknown	adult	Shenyang Forest Zoological Garden
Captive wolf 11	Captive wolf II	Captive	SRR13765885	unknown	adult	Changchun Plant and Animal Park
Captive wolf 12	Captive wolf II	Captive	SRR13765884	unknown	adult	Changchun Plant and Animal Park
Captive wolf 13	Captive wolf II	Captive	SRR13765883	unknown	adult	Changchun Plant and Animal Park
Wild red fox 1	Red fox	Wild	SRR12261820	unknown	unknown	Hulun Lake area
Wild red fox 2	Red fox	Wild	SRR12261821	unknown	unknown	Hulun Lake area
Wild red fox 3	Red fox	Wild	SRR12261822	unknown	unknown	Hulun Lake area
Wild corsac fox 1	Corsac fox	Wild	SRR12261817	unknown	unknown	Hulun Lake area
Wild corsac fox 2	Corsac fox	Wild	SRR12261818	unknown	unknown	Hulun Lake area
Wild corsac fox 3	Corsac fox	Wild	SRR12261819	unknown	unknown	Hulun Lake area
Domestic dog 1	Domestic dog I	Captive	SRR7221649	male	adult	Hulun Lake area
Domestic dog 2	Domestic dog I	Captive	SRR7221650	female	adult	Hulun Lake area
Domestic dog 3	Domestic dog I	Captive	SRR7221651	male	adult	Hulun Lake area
Domestic dog 4	Domestic dog I	Captive	SRR21169620	female	adult	Hulun Lake area
Domestic dog 5	Domestic dog I	Captive	SRR21169621	male	adult	Hulun Lake area
Domestic dog 6	Domestic dog I	Captive	SRR21169622	male	adult	Hulun Lake area
Domestic dog 7	Domestic dog I	Captive	SRR21169628	female	adult	Hulun Lake area
Domestic dog 8	Domestic dog II	Captive	SRR14202219	female	adult	southern China
Domestic dog 9	Domestic dog II	Captive	SRR14202243	male	underage	southern China
Domestic dog 10	Domestic dog II	Captive	SRR14202214	male	adult	southern China
Domestic dog 11	Domestic dog II	Captive	SRR14202216	female	adult	southern China
Domestic dog 12	Domestic dog II	Captive	SRR14202239	male	adult	southern China
Domestic dog 13	Domestic dog II	Captive	SRR14202207	male	underage	southern China
Domestic dog 14	Domestic dog II	Captive	SRR14202254	female	adult	southern China

ORFs (meta-mode) [36]. Based on quantitative ORF results, KEGG abundance tables (levels 1–4) were generated using EggNOG-mapper (V 2.1.10) and the EggNOG database (V 5.0.2). We used Diamond (V 2.0.15) for blast searches of the non-redundant gene catalog against the Carbohydrate-Active enZymes (CAZymes) database (CAZy, V 2023-12; e-value cutoff of 1e-5) and Virulence Factor Database (VFDB, http://www.mgc.ac.cn/VFs/; e-value cutoff of 1e-5). Candidate genes were obtained through filtering based on e-values, and the abundances of CAZy and VFDB were obtained for each sample (the code and statistical methods were from EasyMetagenome; https://github.com/YongxinLiu/EasyMetagenome). The 'MMUPHin' package in R software (V 4.4.1) was used to correct for batch effects on the relative abundance table (species, metabolic pathway, carbohydrate-active enzyme family, and virulence factor) obtained from the above analysis.

Based on the largest abundance (top 10) of the phyla, species, metabolism pathway, carbohydrate-active enzyme family, and virulence factor, a relative abundance column cumulative plot was generated using the 'ggplot2' package in R package (V 4.4.1). The 'vegan' package in R software (V 4.4.1) was used for Adonis (nonparametric MANOVA, $R^2 > 0$, $p < 0.05$; Bray–Curtis and Jaccard distances). Adonis results based on Bray–Curtis and Jaccard distances were largely consistent; accordingly, we only used Bray–Curtis distances for a principal coordinate analysis (PCoA) using OmicShare tools (https://www.omicshare.com/tools).

Alpha diversity can represent the gut microbiome quantity (Observed species index) and species diversity

(Shannon index) of CAZymes and VFs. Briefly, VFs are substances expressed or secreted by pathogens that are related to pathogenicity [37], and CAZymes are responsible for the degradation of carbohydrates. Accordingly, we estimated the pathogenic potential of the gut microbiome in wolves and domestic dogs in captive environments based on VF diversity (Shannon and Simpson indices). Similarly, the ability of the canine gut microbiota to decompose carbohydrates was evaluated through a carbohydrate diversity assessment. The Observed species and Shannon indices of CAZymes and VFs were calculated using the 'vegan' package in R software (V 4.4.1). The 'ggplot2' package in R software (V 4.4.1) was used to generate boxplots of intergroup differences in the Observed species and Shannon (Mann–Whitney U test, $p \le 0.05$). Species and metabolic pathway differences were analyzed using the 'ALDEx2' and 'DESeq2' packages in R software (V 4.4.1), respectively. The differential microbial species and metabolic pathways screened using ALDEx2 ($|\text{effect}| \ge 3$, $p < 0.01$) and DESeq2 ($|\log_2 FC| \ge 1$, $p < 0.05$) may not necessarily be related to ecological niches. Therefore, based on the results of ALDEx2 and DESeq2 analyses, we fitted the correlations between spatial niche width values (wild wolves, 0.5051; red foxes, 0.4292; corsac foxes, 0.2591) and the gut microbiome (the abundance of bacterial species and pathways) of canids using the "randomForest" package in R software (V 4.4.1). Based on environmental factors (e.g., shelter, disturbances, and water availability) and the number of canid dens, our laboratory has used the Shannon–Weiner diversity index [38] to calculate the niche breadth of wild canids by SPSS (V 12.0), and the results are published in the ACTA Ecologica Sinica (Chinese journal) [17].

Results

Data statistics

After quality control and removal of host sequences, we obtained a total 255.62 Gb effective data. The assembled sequence count was 3,456,942 contigs (≥ 500 bp) with a total length of 4.25 Gb. A total of 6,588,205 open reading frames (ORFs) were obtained with a total length of 3.27 Gb using the prodigal software.

Gut microbiome composition

Overall, 4 kingdoms, 89 phyla, 118 classes, 170 orders, 760 families, 2,616 genera, and 10,466 species were detected in the gut microbiome of 36 samples. The proportions of archaea, bacteria, eukaryotes, and viruses were 0.05–0.15%, 82.36–99.94%, 0.06–17.34%, and 0.03–0.85%, respectively. At the phylum level, the relative abundances of Firmicutes (6.07–77.54%) and Bacteroidetes (13.97–77.99%) were highest in the gut microbiome of canids (Fig. 1A). At the species level (Fig. 1B), *Bacteroides sartorii* was the most abundant in captive wolf (I and II; 2.29–20.83%), wild wolf (5.12–6.41%), and corsac fox (1.98–7.61%). *Megalodesulfovibrio gigas* was the most abundant in domestic dog I (0.24–47.73%) and domestic dog II (0.08–15.74%). *Phobpsivirus agricola* was dominant in red fox (5.65–8.91%). As shown in Figure S1, transporter (5.01–6.91%), GH2 (glycoside hydrolase family 2; 4.13–7.84%), and Capsule (8.72–12.75%) were the most abundant metabolic pathway, carbohydrate-active enzyme family, and virulence factor in all groups, respectively.

Cluster analyses

The gut microbiomes (bacterial species, metabolic pathway, carbohydrate-active enzyme family, and virulence factor profiles) of captive wolf I and II were highly similar despite different captive environments, similar to results for domestic dog I and II (Figs. 2 and 3).

Adonis indicated that the captive environment type had a relatively small impact on domestic dogs and captive wolves (Table 2, $0.002 \ge R^2 \le 0.026$, $0.924 \ge p \le 1$; Table 3, $0.002 \ge R^2 \le 0.026$, $0.493 \ge p \le 1$). Therefore, we merged data for domestic dog I and domestic dog II into one group (domestic dog) and merged data for captive wolf I and captive wolf II into one group (captive wolf). There were no significant differences between sexes in the composition of the gut microbiome, metabolic pathways, carbohydrate–active enzymes, and virulence factors in captive wolf and domestic dog (Table 2, $0.065 \ge R^2 \le 0.326$, $0.097 \ge p \le 0.646$; Table 3, $0.051 \ge R^2 \le 0.326$, $0.083 \ge p \le 0.818$). Furthermore, data for only two young individuals (two months old) were included, and age was also not a major influencing factor (Table 1).

At the species level (Fig. 2A; Table 2, and Table 3), wild and captive wolves (species level; Table 2, $R^2 = 0.079$, $p = 0.273$; Table 3, $R^2 = 0.079$, $p = 0.266$) exhibited greater similarity than that of the captive wolf and domestic dog (species level; Tables 2 and 3, $R^2 = 0.182$, $p = 0.001$). However, the metabolic pathway compositions of captive wolf and domestic dog were more similar (Tables 2 and 3; Figs. 2B and 3B); Adonis results (Tables 2 and 3) showed that the metabolic pathway composition of captive wolf was more similar to that of wild wolf than domestic dog. Based on the relative abundances of carbohydrate-active enzyme families (Figs. 2C and 3C) and virulence factors (Figs. 2D and 3D), the distances between captive wolf and domestic dog were closer than those between captive wolf and wild wolf (Tables 2 and 3). The compositions of bacterial species, metabolic pathways, carbohydrate-active enzyme families, and virulence factors in wild wolves, red foxes, and corsac fox were clearly separated (Figs. 2 and 3; Table 2, and Table 3).

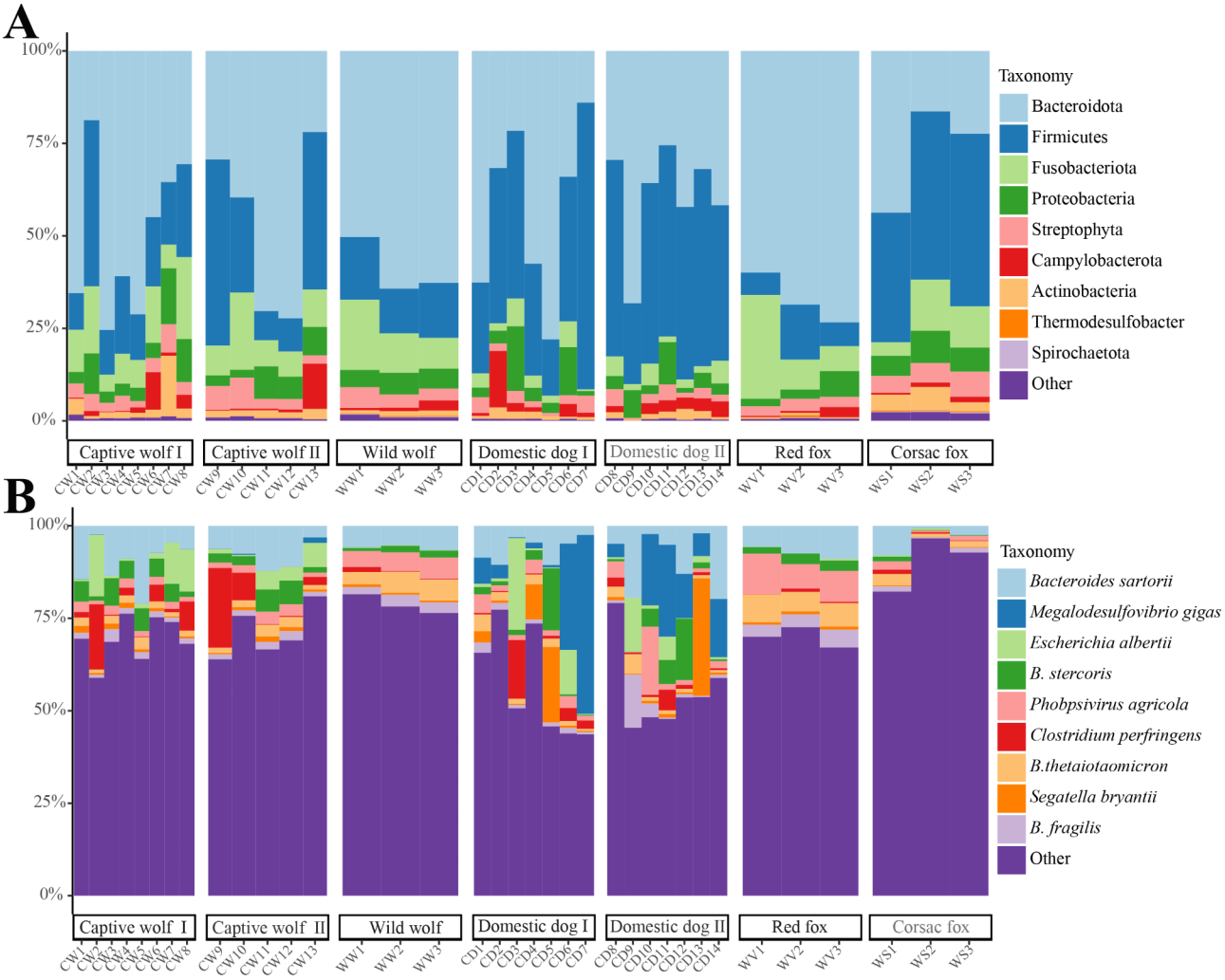

Fig. 1 The relative abundance plot of the top ten phylum (**A**) and species (**B**)

Alpha diversity difference analysis

Observed species and Shannon indices represent the gut microbiome quantity and diversity, respectively. Compared with those in wild wolf and corsac fox, the quantity and diversity of CAZymes were higher in red fox (red fox vs. corsac fox, Observed species index: $p > 0.05$, Shannon index: $Z = 1.96$, $p = 0.05$; red fox vs. wild wolf, Observed species and Shannon indices: $Z = 1.96$, $p = 0.05$; Figure S2A and S2B). The quantity and diversity of CAZymes were highest in domestic dog (domestic dog vs. captive wolf, Observed species index: $Z = 2.48$, $p = 0.01$, Shannon index: $p > 0.05$; domestic dog vs. wild wolf, observed species and Shannon indices: $Z = 2.65$, $p = 0.008$), followed by captive wolf and were lowest in wild wolf (captive wolf vs. wild wolf, observed species and Shannon indices: $Z = 2.63$, $p = 0.008$; Fig. 3A and 3).

The quantity and diversity of VFs were significantly higher in red fox than in wild wolf and corsac fox (red fox vs. corsac fox, Observed species and Shannon indices: $Z = 1.96$, $p = 0.05$; red fox vs. wild wolf, Observed species and Shannon indices: $Z = 1.96$, $p = 0.05$; Figure S2C and S2D). The quantity and diversity of VFs were highest in domestic dog (domestic dog vs. captive wolf, Observed species and Shannon indices: $p > 0.05$; domestic dog vs. wild wolf, Observed species index: $Z = 2.39$, $p = 0.02$, Shannon index: $Z = 2.02$, $p = 0.04$), followed by captive wolf. The quantity and diversity of VFs were lowest in wild wolf (captive wolf vs. wild wolf, observed species index: $p > 0.05$, Shannon index, $Z = 1.95$, $p = 0.05$; Fig. 3C and 3).

Gut microbiome associated with spatial niche differentiation

We detected 312 differential bacterial species (Supplementary Table 1) and 72 pathways (Supplementary Table 2) between wild canids (wild wolf vs. red fox; wild wolf vs. corsac fox; red fox vs. corsac fox) using ALDEx2 ($|\text{effect}| \geq 3$, $p < 0.01$) and DESeq2 ($|\log_2 FC| \geq 1$, $p < 0.05$),

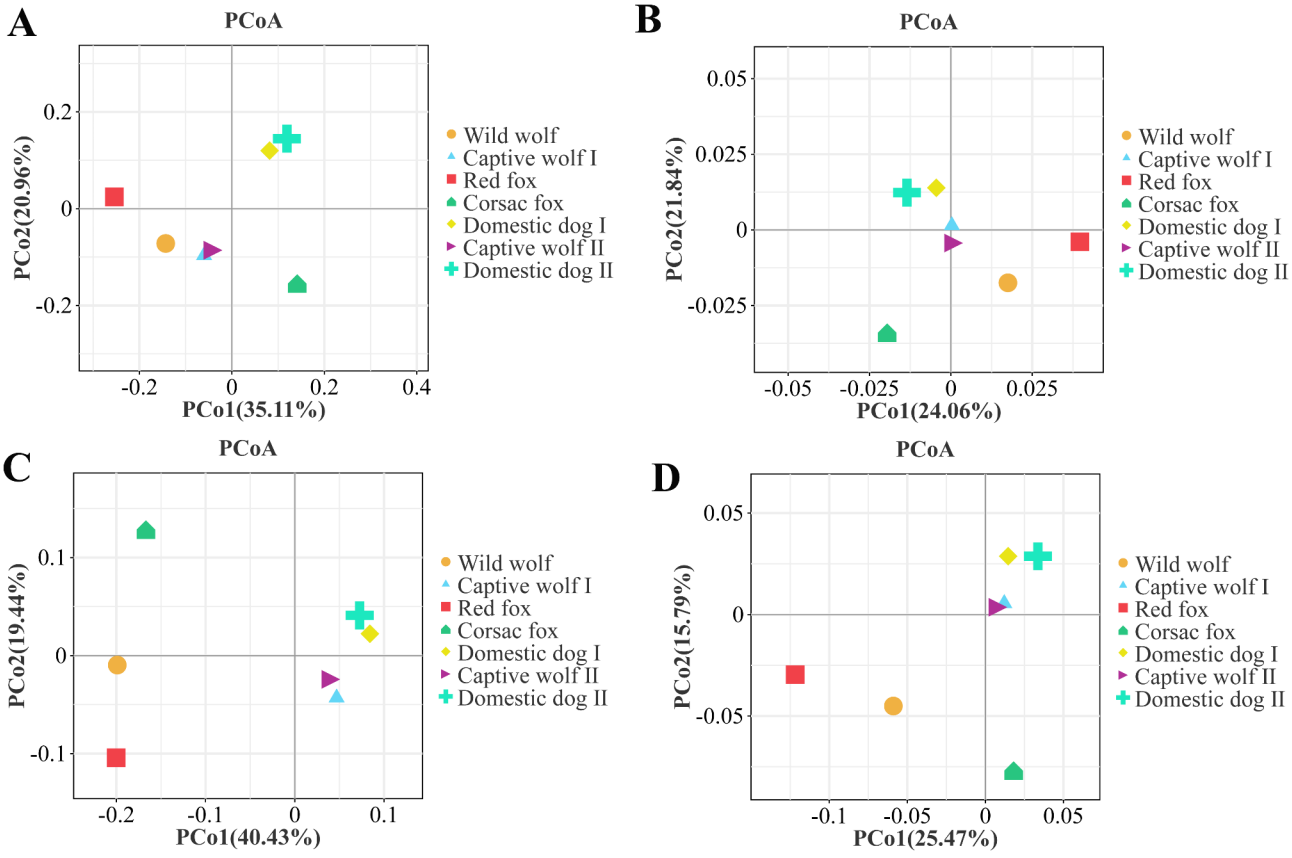

Fig. 2 Principal coordinates analysis (PCoA) plots based on Bray–Curtis distances. (**A**) Bacterial species, (**B**) metabolic pathways, (**C**) carbohydrate-active enzyme families, and (**D**) virulence factors

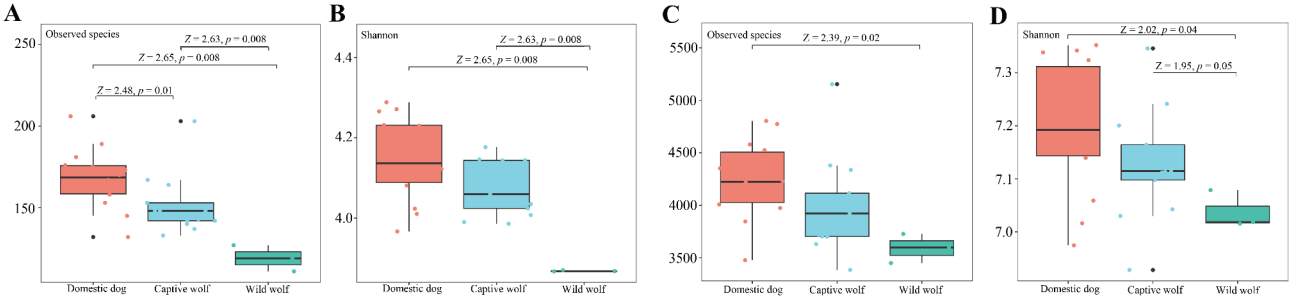

Fig. 3 Box plots of alpha-diversity in wild wolf, captive wolf, and domestic dog and comparisons using Mann–Whitney U tests. Observed species (**A**) and Shannon (**B**) indices of carbohydrate-active enzymes and observed species (**C**) and Shannon (**D**) indices of virulence factor diversity are shown

respectively. Based on these results, 19 potential bacterial species (Fig. 4A; Table 4) and 20 potential metabolic pathways (Fig. 4B; Table 4) were significantly positive associated with spatial niche differentiation (Spatial niche width index: wild wolf, 0.5051; red fox, 0.4292; corsac fox, 0.2591; $p < 0.05$) through a random forest analysis.

Differences between wild wolf, captive wolf, and domestic dog

We detected 23 bacterial species (Supplementary Table 3) differing in relative abundance between wild wolf, captive wolf, and domestic dog (wild wolf vs. captive wolf; wild wolf vs. domestic dog; captive wolf vs. domestic dog) using ALDEx2 (|effect| ≥ 3, $p < 0.01$). Twenty-two bacterial species were enriched in wild wolf, and the abundance of *Turicibacter sp.* H121 was significantly increased in captive wolves and dogs. In comparisons with wild wolf, captive wolf and domestic dog shared one upregulated metabolic pathway (ko05166, Human T-cell leukemia virus 1 infection; Supplementary Table 4).

Table 2 Adonis analyses with Bray-Curtis distance

Group	Description	R^2	F	*p*
Domestic dog I / Domestic dog II (Different captive environments)	Species	0.026	0.323	0.959
	KEGG	0.024	0.296	0.986
	CAZy	0.017	0.208	0.998
	VFDB	0.0093	0.071	0.924
Captive wolf I / Captive wolf II(Different captive environments)	Species	0.005	0.060	1
	KEGG	0.012	0.138	1
	CAZy	0.013	0.149	0.997
	VFDB	0.002	0.023	1
Domestic Dog male / Domestic Dog female	Species	0.112	1.515	0.166
	KEGG	0.065	0.834	0.646
	CAZy	0.071	0.919	0.492
	VFDB	0.075	0.966	0.458
Captive Wolf male / Captive Wolf female	Species	0.326	2.901	0.097
	KEGG	0.204	1.537	0.146
	CAZy	0.204	1.535	0.156
	VFDB	0.221	1.700	0.219
Wild wolf / Captive wolf	Species	0.079	1.206	0.273
	KEGG	0.106	1.657	0.190
	CAZy	0.414	9.908	0.001
	VFDB	0.164	2.742	0.012
Captive wolf / Domestic dog	Species	0.182	5.547	0.001
	KEGG	0.117	3.298	0.001
	CAZy	0.132	3.793	0.002
	VFDB	0.106	2.965	0.006
Wild wolf / Corsac fox / Red fox	Species	0.613	4.746	0.015
	KEGG	0.644	5.415	0.007
	CAZy	0.610	4.699	0.011
	VFDB	0.609	4.673	0.009

The larger the R^2 value, the greater the difference between the two groups, and vice versa. If the *p*-value is less than or equal to 0.05, it indicates that there is a significant difference between the groups. F value is mean square/mean square error

Discussion

By comparing metagenomic data for canid species, we found that captive environments drive similarities in the compositions of carbohydrate-active enzymes and virulence factors in the gut microbiome of captive wolf and domestic dog. The captive environment had little impact on the gut bacterial species and metabolic pathway composition of wolf. Genetic factors play a dominant role in shaping bacterial community characteristics and functions [39, 40]. The observed similarity in the composition of bacterial species and metabolic pathways between captive and wild wolves could be attributed to the retention of the natural gut microbiota in captive wolves. Carbohydrate-active enzyme families and virulence factors can be transferred from environmental bacteria to the gut microbiota of animals [41, 42]. This may explain the similar composition of carbohydrate-active enzyme families and virulence factors in the gut microbiomes of captive wolf and domestic dog as well as the increased diversity of carbohydrate-active enzyme families and virulence factors in the gut microbiome of captive wolf. VFs are substances that are expressed or secreted by pathogens that are related to pathogenicity [37]. Therefore, the increase in VF diversity may lead to an increase in the pathogenic potential of the gut microbiome in captive wolves. Furthermore, Human T-cell leukemia virus 1 infection was significantly enriched in the gut microbiomes of captive wolf and domestic dog. Ramassamy et al. have identified the zoonotic transmission of Human T-Cell Leukemia Virus-1 among humans and animals in Central Africa [43]. However, we did not measure the abundance of Human T-Cell Leukemia Virus-1 in the guts of feeders and therefore its transmission from humans to role in captive wolves and domestic dogs is unclear and requires further experimental verification.

Among the three wild canids evaluated in this study, the corsac fox had the smallest spatial niche and arid environment [17, 28]. Corsac fox had a distinct gut microbiome from those of red fox and wild wolf. Maintaining physiological homeostasis and health is critical for corsac fox survival in arid environment. A random forest analysis screened bacterial species related to the ecological niche of corsac foxes, all of which are associated with maintaining physiological homeostasis and

Table 3 Adonis analyses with Jaccard distance

Group	Description	R^2	F	*p*
Domestic dog I vs. Domestic dog II (Different captive environments)	Species	0.026	0.323	0.961
	KEGG	0.024	0.296	0.996
	CAZy	0.011	0.145	1
	VFDB	0.071	0.924	0.493
Captive wolf I vs. Captive wolf II(Different captive environments)	Species	0.005	0.060	1
	KEGG	0.012	0.138	1
	CAZy	0.013	0.149	0.999
	VFDB	0.002	0.023	1
Domestic Dog male vs. Domestic Dog female	Species	0.093	1.224	0.267
	KEGG	0.051	0.644	0.818
	CAZy	0.075	0.967	0.401
	VFDB	0.059	0.756	0.743
Captive Wolf male vs. Captive Wolf female	Species	0.326	2.901	0.083
	KEGG	0.204	1.537	0.143
	CAZy	0.204	1.535	0.160
	VFDB	0.221	1.700	0.214
Wild wolf vs. Captive wolf	Species	0.079	1.206	0.266
	KEGG	0.106	1.657	0.183
	CAZy	0.414	9.908	0.004
	VFDB	0.164	2.742	0.013
Captive wolf vs. Domestic dog	Species	0.182	5.547	0.002
	KEGG	0.117	3.298	0.004
	CAZy	0.132	3.793	0.001
	VFDB	0.106	2.965	0.003
Wild wolf vs. Corsac fox vs. Red fox	Species	0.613	4.746	0.008
	KEGG	0.644	5.415	0.004
	CAZy	0.610	4.700	0.005
	VFDB	0.609	4.673	0.004

The larger the R^2 value, the greater the difference between the two groups, and vice versa. If the *p*-value is less than or equal to 0.05, it indicates that there is a significant difference between the groups. F value is mean square/mean square error

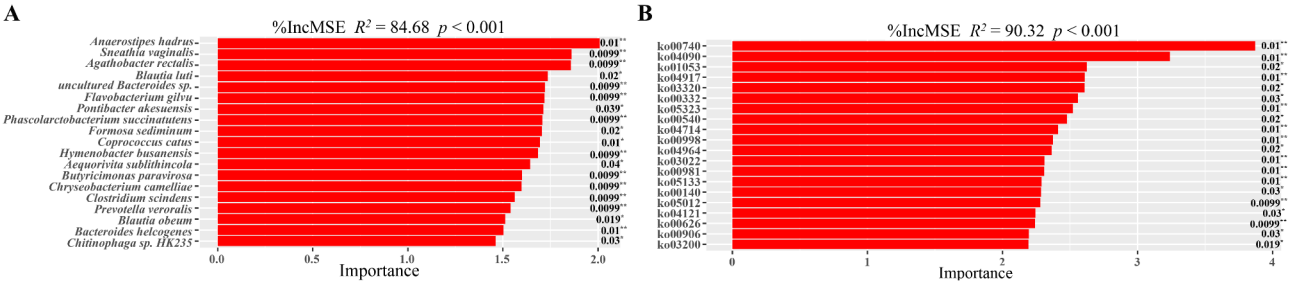

Fig. 4 Bar plots based on random forest models of potential bacteria species (**A**) and pathways (**B**) with spatial niche differentiation. R^2, total variance explained. *p*, significance of the entire model. After each bar, the *p*-value for each bacteria species or pathway is shown. %IncMSE, percentage of increase of mean square error

health [18, 44–47]. For example, *Clostridium scindens* participates in the regulation of the metabolic pathway ko00998 to generate multiple antibiotics that can maintain gut homeostasis and host health [11]. The metabolic pathway ko04964 promotes the recycling of bicarbonate and sodium ions by host proximal tubules, thereby maintaining acid-base and osmolality homeostasis [48, 49]. The significant enrichment of the metabolic pathway ko04964 may contribute to the adaptation of the corsac fox to arid environments.

The spatial ecological niche of red fox is larger than that of corsac fox and smaller than that of wild wolf; the species typically occupies caves located in willow bosk [17]. Some studies have found that plant seeds (containing carbohydrates) are among the main constituents of red fox diets, such as Poaceae and Rosaceae, which are abundant in willow bosk [13, 50, 51]. The dominant bacteria in the

Table 4 Potential bacterial species and metabolic pathways associated with Spatial niche differentiation of wild Wolf, red Fox, and corsac Fox

Species	Bacterial species	Metabolic pathway
Wild wolf	uncultured *Bacteroides sp.*, *Bacteroides helcogenes*, *Chryseobacterium camelliae*, *Chitinophaga sp.* HK235, *Flavobacterium gilvu*, *Formosa sediminum*, *Hymenobacter busanensis*, *Phascolarctobacterium succinatutens*, *Prevotella veroralis*, and *Sneathia vaginalis*	ko00740, Riboflavin metabolism; ko04090, CD molecules; ko03320, PPAR signaling pathway; ko05323, Rheumatoid arthritis; ko00540, Lipopolysaccharide biosynthesis; ko04714, Thermogenesis; ko05133, Pertussis
Red fox	*Aequorivita sublithincola*, *Butyricimonas paravirosa*, and *Pontibacter akesuensis*	ko01053, Biosynthesis of siderophore group nonribosomal peptides; ko00140, Steroid hormone biosynthesis; ko03200, Viral proteins
Corsac fox	*Agathobacter rectalis*, *Anaerostipes hadrus*, *Blautia luti*, *Blautia obeum*, *Clostridium scindens*, and *Coprococcus catus*	ko04917, Prolactin signaling pathway; ko00332, Carbapenem biosynthesis; ko00998, Biosynthesis of various secondary metabolites - part 2; ko04964, Proximal tubule bicarbonate reclamation; ko03022, Basal transcription factors; ko00981, Insect hormone biosynthesis; ko05012, Parkinson disease; ko04121, Ubiquitin system; ko00626, Naphthalene degradation; ko00906, Carotenoid biosynthesis

gut microbiome of red fox included carbohydrate decomposition specialists, such as *Bacteroides sartorii* [52], *B. sthetaiotaomicron* [53], and *Butyricimonas paravirosa* [54]. Compared with those of corsac fox and wild wolf, the greater alpha diversity (carbohydrate-active enzyme family) in the gut microbiome of red fox may reflect the stronger ability to break down carbohydrates in foods. Red fox had a significantly higher diversity of virulence factors than that of wild wolf, and a higher diversity of virulence factors than that of the corsac fox (though the difference was not significant). Virulence factors play a pivotal role in the infectious process by determining the pathogenic potential of microorganisms and can directly interact with host tissues or facilitate bacterial evasion of the immune response [55, 56]. In addition, viral proteins (ko03200) were significantly enriched in the gut of red foxes. Although there is a correlation between viral proteins and spatial ecological niches, viral infection is also related to animal species [57–59]. Therefore, we believe that the differences in viral protein abundance are related to differences in the spatial niche and host.

Compared with those of red and corsac fox, wild wolf occupies a wider spatial ecological niche [17]. This broader spatial niche means that wild wolves require more energy for biological activities [60]. Bacterial species producing SCFAs were significantly enriched in the gut microbiome of wild wolf (compared with red and corsac fox), including *Prevotella veroralis* [61, 62] and *Phascolarctobacterium succinatutens* [63]. SCFAs play an important role in maintaining health and providing energy to the host [64, 65]. The metagenomes of wild wolf (compared with red and corsac fox) were enriched for metabolic pathways associated with the production of SCFAs, including Riboflavin metabolism [66] and PPAR signaling pathway [67]. Thermogenesis was also enriched in metagenomes of wild wolf, which may utilize SCFAs to provide energy [68]. However, it is necessary to validate the bacterial species and metabolic pathways associated with spatial ecological niche differentiation through field experiments and colony transplantation experiments.

Conclusion

In summary, we conducted a preliminary exploration of the differences in the gut microbiomes of canids in different ecological niches and environments. Although, we screened candidate microbial taxa and metabolic pathways of canids related to spatial niche adaptation through a random forest analysis, we cannot rule out the influence of host genetic factors. Accordingly, further validation is needed through microbiome transplantation and host physiological experiments. The captive environment shapes the composition of CAZymes and VFs in the gut microbiota of captive wolves, resulting in similar profiles to those of domestic dogs. In addition, captive

environments increased the diversity of VFs in the gut microbiome of captive wolves, which may increase the pathogenic potential of the gut microbiome. Therefore, it is necessary to continue monitoring the health status of captive wolves and develop appropriate management strategies.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12866-025-03863-2.

Supplementary Material 1: **Supplementary Table 1**: Results of an ALDEx2 analysis of differential bacterial species between wild wolf, red fox, and corsac fox ($|effect| \geq 3$, $p < 0.01$)

Supplementary Material 2: **Supplementary Table 2**: Results of a DESeq2 analysis of differential expression between wild wolf, red fox, and corsac fox ($|\log_2 FC| \geq 1$, $p < 0.05$)

Supplementary Material 3: **Supplementary Table 3**: Results of an ALDEx2 analysis of differential bacterial species between wild wolf, captive wolf, and domestic dog ($|effect| \geq 3$, $p < 0.01$)

Supplementary Material 4: **Supplementary Table 4**: Results of a DESeq2 analysis of differential expression between wild wolf, captive wolf, and domestic dog ($|\log_2 FC| \geq 1$, $p < 0.05$)

Supplementary Material 5: **Figure S1**: Relative abundances of the top ten metabolic pathways (A), CAZymes (B), and VFs (C)

Supplementary Material 6: **Figure S2**: Box plots of alpha-diversity in-wild wolf, red fox, and corsac fox and comparisons using Mann–Whitney U tests. Observed species (A) and Shannon (B) indices of carbohydrate-active enzymes and observed species (C) and Shannon (D) indicesof virulence factor diversity are shown

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Author contributions
X. Wa.: Writing– review & editing, Writing– original draft, Methodology, Conceptualization; Y. S.: Methodology, Data curation; Y. X.: Methodology, Data curation; Y. C. and X. Wu.: Data curation; H. Z.: Writing– review & editing, Methodology, Funding acquisition, Conceptualization.

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Data availability
All metagenomic data are shown in Table 1.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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