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In-Silico and Microbiological Analysis of the Gut Microbiota Composition between Male and Female Tiger under Captive Condition in Patna Zoo

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Abstract : *This project investigates the gut microbiota composition in Bengal tigers, with a comparative analysis between male and female and in-silico comparison between captive individuals and their wild counterparts. Gut microbiota plays a critical role in animal health, influencing digestion, immune function, and overall well-being. For carnivores like tigers, these microbial communities aid in nutrient extraction from protein-rich diets and contribute to resistance against pathogens.*

The study aims to understand how captivity might influence the diversity and structure of the tiger gut microbiota, with implications for tiger conservation and rewilding strategies. Samples were analyzed to assess microbial diversity and composition, potentially revealing insights into dietary influences, habitat-related microbial exposure, and health indicators. This research provides a foundation for understanding the gut microbiota's role in tiger physiology and informs conservation efforts focused on maintaining or restoring natural microbial profiles to support reintroduction and health management strategies for captive populations.

Keywords: *Gut Microbiota, Captive, Bengal Tigers, In-Silico.*

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Introduction:

"All disease begins in the gut"– (*Hippocrates of Kos).*

The gut microbiota is a diverse and highly dense community of microorganisms that reside in the gastrointestinal tract and are implicated in various physiological functions of the host organism including digestion, immune response and metabolism (Ding & Schloss, 2014; Ley, Turnbaugh, et al., 2006; Turnbaugh et al., 2006). The concept of "microbiota" was first introduced by Lederberg and McCray (2001) to describe all microbial entities within a host, encompassing bacteria, archaea, viruses, and eukaryotic microorganisms, each playing distinct roles in biological processes. The gut provides a niche for the growth and proliferation of a diversity of microorganisms. Notably, the gut microbiota contributes to the digestion of complex carbohydrates, the synthesis of certain vitamins, and the modulation of the host's immune system (Hooper et al., 2012) (Clemente et al., 2012).The composition of gut microbiota is largely determined by several intrinsic and extrinsic factors such as the host's environment, health status, genotype, dietary habits, age, sex, social relationships and disease prevalence (Degnan et al., 2012; Ley, Peterson, et al., 2006; Redford et al., 2012; Tung et al., 2015).

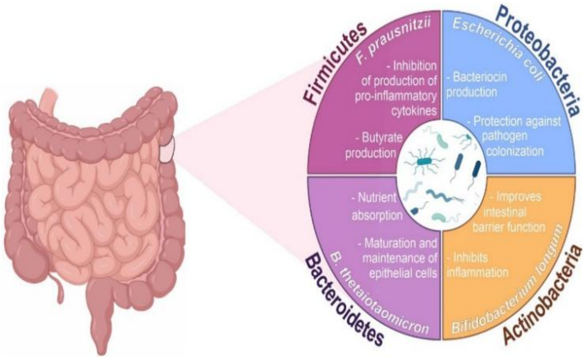

Fig. 1. Overview of the major gut microbiota phyla and their physiological functions

In carnivorous mammals such as tigers, the gut microbiome is primarily composed of bacterial phyla like *Firmicutes, Bacteroidota, Actinobacteriota* and *Proteobacteria* which play essential roles in breaking down complex carbohydrates and proteins. This process leads to the production of metabolites such as short-chain fatty acids, which are essential for energy metabolism and maintaining gut health, **Fig. 1** (He et al., 2018; Ley et al., 2008). The Royal Bengal tiger *(Panthera tigristigris)* is one of the 9 recognized subspecies of tiger *(Panthera tigris),* belonging to the genus *Panthera.* It ranks as one of the largest cat species globally, second only to the Siberian tiger.

Table 1. Comparison of Immune Function Indicators in Tigers with Balanced vs. Dysbiotic Gut Microbiota

Indicator	Balanced Microbiota	Dysbiotic Microbiota
Immune Response Efficiency	High	Low
Infection Occurrence	Low	High
Systemic Inflammation	Minimal	Elevated
Antimicrobial Peptides	Increased	Decreased
Gut Barrier Function	Stable	Weakened

Factors Influencing Gut Microbiota Composition: Captive environments, such as rehabilitation centres, breeding facilities and zoos induce significant changes in the gut microbiota of tigers compared to their natural habitats. These changes are driven by factors like diet, medical interventions (e.g., antibiotics), restricted movement, reduced exposure to diverse habitats and increased interaction with human-associated microbes *(Hyde et al., 2016).*

Fig. 2. Accountability of different factors affecting the gut microbiota of tigers

However, diet plays a central role in shaping the gut microbiota of tigers. Wild tigers consume a varied diet, including prey, bones, organs and skin which promotes a diverse and healthy microbial flora (Barton et al., 2014). However, captive tigers are often fed a standardized diet that lacks this diversity leading to a decrease in microbial diversity and potentially contributing to metabolic issues like obesity and gastrointestinal disorders (Schmidt et al., 2018).

The geographic location also impacts the gut microbiota composition. Siberian tigers, adapted to colder climates, have gut microbiota that aids in fat digestion, unlike Bengal tigers that inhabit warmer regions and consume different prey (Cresci & Bawden, 2015).

The health conditions and stress significantly affect the gut microbiota in tigers, particularly in captive individuals. Illness and stress can induce dysbiosis, characterized by a decrease in beneficial bacteria and an increase in harmful ones (Deng et al., 2021). This imbalance can exacerbate gastrointestinal disorders and immune deficiencies, highlighting the importance of maintaining a healthy gut microbiome for overall well-being.

In conclusion, the gut microbiota of captive tigers is influenced by dietary restrictions, environmental changes, and health conditions. These factors affect their overall health, immune function and susceptibility to diseases.

Sanjay Gandhi Biological Park, (Patna Zoo), is located in Patna, Bihar, India. Established in 1969, the zoo is known for its significant population of Bengal tigers. The zoo's tiger conservation efforts began in 1975 with the arrival of a male tiger named Moti from Delhi Zoo. In 1980, two tigresses from Assam, Bulborani and Fauzi were introduced, marking the start of the zoo's tiger breeding program. The first tiger cub, a female, was born on January 1, 1983, from Bulborani. Today, Patna Zoo is home to four white Bengal tigers and two regular Royal Bengal Tigers underscoring its ongoing commitment to tiger conservation and breeding.

Gut Microbiota and Conservation of Tigers: Tigers, listed as endangered by the International Union for Conservation of Nature (IUCN), face significant threats from illegal trade and habitat loss highlighting the urgent need for conservation efforts. Captive tigers, unlike their wild counterparts, often suffer from altered gut microbiota due to a less diverse diet. This alteration can lead to health issues such as obesity, diabetes, and reduced lifespan, as seen in studies of captive animals (Schmidt et al., 2018). Wild tigers with their varied diet maintain a healthier and more diverse gut microbiota. Research has shown that captive tigers have lower microbial diversity making them more susceptible to diseases and less adaptable to environmental changes.

Studies of Bengal tiger gut bacteria have revealed important bacterial groups like *Bacteroidetes* that play a crucial role in maintaining health. Comparisons between male and female, and captive vs. wild tigers, are necessary to understand how captivity impacts their microbiota. The use of microbial and carnivore databases allows for comparisons between wild and captive tiger gut microbiota.

This research aims to investigate the impact of captivity on tiger gut microbiota by comparing fecal samples from male and female tigers at Patna Zoo with data from wild tigers. The goal is to understand how captivity alters microbial diversity and composition, identify health implications, and contribute to improving captive tiger health and welfare. The findings will support conservation strategies for both captive and wild tigers.

Material and Methods:

Chemicals: Peptone, Beef extract, Sodium chloride, Agar, Bile salts, Neutral Red Solution (1% aqueous), Crystal Violet, Distilled water, Trypticase (animal Peptone), Phytone (soy Peptone), Tryptose, Dextrose, Yeast Extract, Proteose Peptone, Lactose Monohydrate, Neutral Red, Ethanol, Tryptone, Amyl Alcohol, Bromothymol blue, P-Dimethylaminobenzaldehyde, Phenol red, VPI and VP2 reagent, Potassium nitrate, Ammonium dihydrogen phosphate, Dipotassium phosphate, Hydrogen peroxide.

Media and Reagents: Nutrient Agar (NA), MacConkey Agar (MCA), Tryptic Soy Agar (TSA), Blood Agar (BA) and Beef Peptone Agar (BPA) Media, MR-VP broth, Indole broth, Kovac's reagent, Normal saline.

Methods:

Sample Collection: Fecal samples were collected from two adult Bengal tigers (one male and one female) at Patna Zoo stored in sterile containers and transported to the laboratory. Soil samples were taken from the tiger enclosure ensuring no contamination from fecal matter to assess the baseline microbial population.

Media Preparation: 5 culture media were prepared: Nutrient Agar (NA), MacConkey Agar (MCA), Tryptic Soy Agar (TSA), Blood Agar (BA) and Beef Peptone Agar (BPA). Each medium was sterilized by autoclaving at 121°C for 15 minutes and poured into sterile petri dishes.

Serial Dilution and Plating: Fecal and soil samples (0.5 gm) were suspended in 4.5 ml sterile saline. Serial dilutions were performed up to 10^{-5} for the male & soil samples and 10^{-3} for the female tiger sample. 100 µL from each dilution was spread on the prepared agar plates and incubated at 37°C for 24 hours.

CFU Count: Colony-forming units (CFUs) were calculated for male and female tiger fecal samples (10^{-5} and 10^{-3} dilutions, respectively) and soil (10^{-5} dilution) using the agar plates.

Gram Staining: Bacterial colonies were Gram-stained for identification. Colonies from each medium (NA, MCA, TSA, BA, BPA) were picked and subjected to Gram's stain followed by microscopic examination.

pH Measurement: The 0.5 gm of fecal matter from both male and female tigers was mixed with distilled water and the pH was measured using a pH meter. A control sample containing only distilled water was also measured.

Biochemical Characterization:

Catalase Test: Bacterial cultures were tested by placing 5% H_2O_2 on a slide. The presence of gas bubbles indicated catalase activity.

Oxidase Test: A colony was smeared onto filter paper soaked in 1% Kovacs oxidase reagent. A colour change to dark purple within 10 seconds indicated oxidase positivity.

Indole Test: Tryptone broth was inoculated with the test organism and incubated at 37°C for 48 hours. After adding Kovac's reagent, a colour change indicated the presence of indole.

Methyl Red and Voges-Proskauer Test: MR-VP broth was inoculated, incubated at 37°C for 48 hours, and tested with methyl red and VP reagents to check for acidic and neutral fermentation products respectively.

Result and Discussion:

Sample Information of Captive Bengal Tigers at Patna Zoo: Fecal samples were collected from two adult Bengal tigers (one male, one female) at Patna Zoo. Soil samples were also collected from areas in the tiger enclosure free of fecal contamination to assess baseline microbial populations in the zoo environment.

Table 2. Detailed Sample Information of Captive Bengal Tigers at Patna Zoo

Sample No.	Gender	Age (Months)	Weight (kg)	Subspecies	Diet	Sampling Date	Collection Location
1	Male	26	80 kg approx.	Bengal Tiger (*Panthera tigristigris*)	Beef, Goat Meat	16-08-2024	Patna Zoo
2	Female	24	75 kg approx.	Bengal Tiger (*Panthera tigristigris*)	Beef, Goat Meat	16-08-2024	Patna Zoo

Serial Dilution: Serial dilution was performed on fecal samples and soil samples to isolate bacterial colonies. Different dilutions (10^{-5} for male tiger feces and for soil and 10^{-3} for female tiger feces) facilitated colony separation on media plates.

Bacterial Colonies on Media Plates: Bacterial growth was observed on NA, MCA, BA, BPA and TSA. Different bacterial colonies grew at various dilutions, demonstrating the microbial diversity present in the samples.

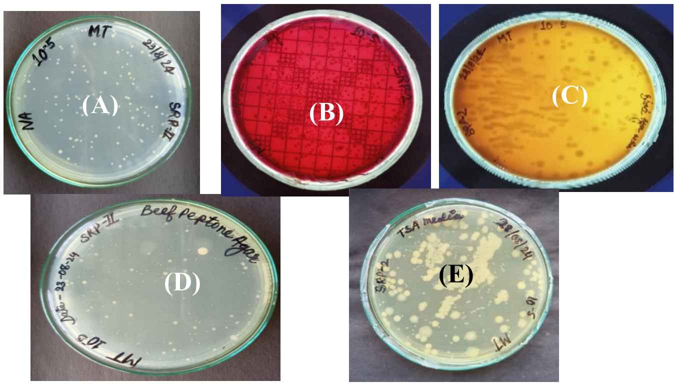

Fig. 3. Bacterial colony growth from male tiger fecal sample at 10^{-5} dilution on *(A) Nutrient Agar, (B) MacConkey Agar, (C) Blood Agar, (D)Beef peptone Agar and (E) Tryptose Soy Agar.*

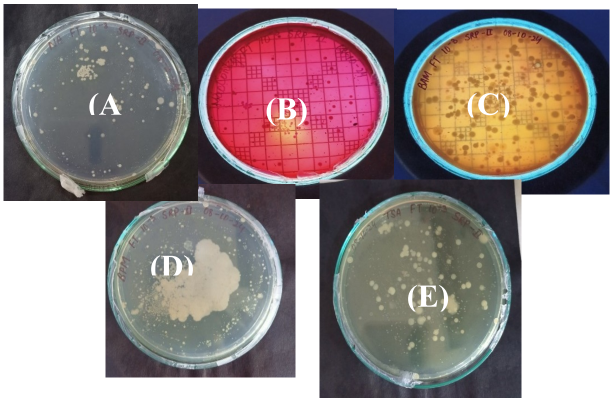

Fig. 4. Bacterial colony growth from female tiger fecal sample at 10^{-3} dilution on *(A) Nutrient Agar, (B) MacConkey Agar, (C) Blood Agar, (D) Beef Peptone Agar and (E) Tryptose Soy Agar.*

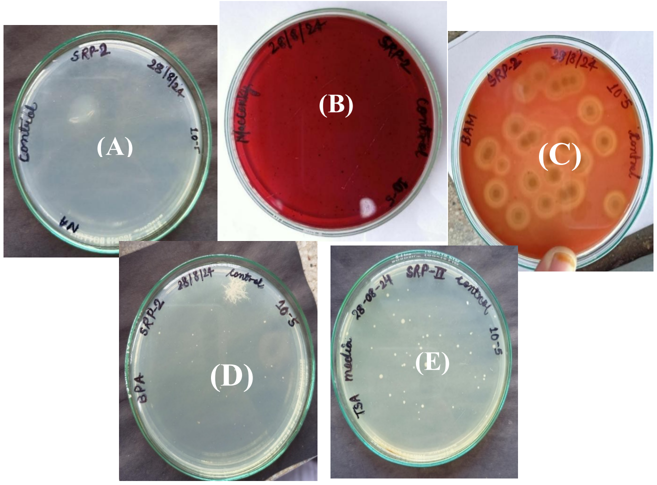

Fig. 5. Bacterial colony growth from soil sample (control) at 10^{-5} dilution on ***(A) Nutrient Agar, (B) MacConkey Agar, (C) Blood Agar, (D) Beef Peptone Agar and (E) Tryptose Soy Agar***

Identification and Colony-Forming Unit (CFU) Count: CFU counts for male and female tiger fecal samples at 10^{-5} and 10^{-3} dilution, and for the soil sample at 10^{-5} dilution, are summarized in Table 3.

Table 3. CFU Counts for Male Tiger, Female Tiger and Soil (Control) Samples Across Various Media Types

Media Type	Male Tiger Fecal Sample	Female Tiger Fecal Sample	Soil (Control) Sample
Nutrient Agar (NA)	108 CFU	49 CFU	5 CFU
MacConkey Agar (MCA)	153 CFU	40 CFU	No bacterial colony
Tryptose Soy Agar (TSA)	158 CFU	44 CFU	41 CFU
Blood Agar (BA)	125 CFU	120 CFU	23 CFU
Beef Peptone Agar (BPA)	189 CFU	103 CFU	26 CFU

Morphological Characterization: Gram staining and morphological analysis were conducted on bacterial strains isolated from all samples. Colonies were selected based on distinct morphological features from various media types.

Tiger gut pH measurement: The gut pH of the male tiger was 6.43, while the female tiger's gut pH was slightly lower at 6.23. The lower pH in the tiger's guts indicates a more acidic environment, typical for carnivores to aid in digesting protein-rich diets.

Biochemical Tests Results: Biochemical tests were performed on bacterial strains isolated from the fecal samples of both male and female tigers, as well as from the control soil sample. These tests provided essential information to identify and differentiate bacterial species offering insights into the microbial composition of the tiger gut and the surrounding environment.

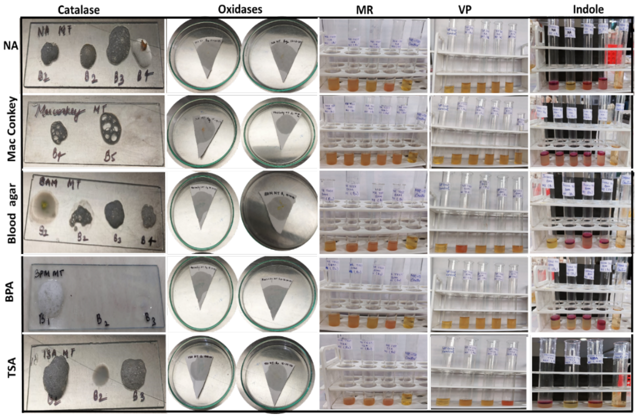

Fig. 6. Biochemicals test results of bacterial strains from male tiger fecal sample on different media's (NA, MacConkey, Blood Agar, Beef Peptone Agar, TSA)

Table 4. Biochemical Test Results for Bacterial Strains Isolated from Male Tiger Fecal Sample on Different Media

Media	Colony Name	Catalase Test	Oxidase Test	Indole Test	MR	VP
NA	B1	+	-	+	+	-
	B2	+	-	-	+	-
	B3	+	-	+	-	-
	B4	-	-	+	+	-
MCA	B1	+	-	+	+	-
	B2	+	-	+	+	-
	B3	+	-	+	+	-
	B4	+	-	+	+	-
	B5	+	-	+	+	-
BAM	B1	+	-	-	-	-
	B2	+	-	+	+	+
	B3	+	-	+	+	-
	B4	+	-	+	+	-
BPA	B1	+	-	+	+	-
	B2	-	-	+	+	-
	B3	-	-	+	-	-
TSA	B1	+	-	+	+	-
	B2	+	-	-	+	-
	B3	+	-	+	+	-

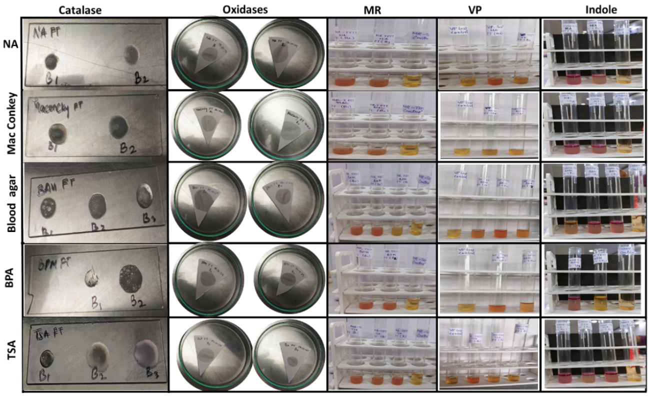

Fig. 7. Biochemicals test results of bacterial strains from female tiger fecal sample on different media's (NA, MacConkey, Blood Agar, Beef Peptone Agar and TSA)

Table 5. Biochemical Test Results for Bacterial Strains Isolated from Female Tiger Fecal Sample on Different Media

Media	Colony Name	Catalase Test	Oxidase Test	Indole Test	MR	VP
NA	B1	+	-	+	+	-
	B2	+	-	+	+	+
MCA	B1	+	-	+	+	-
	B2	+	-	+	+	-
BAM	B1	+	-	-	+	-
	B2	+	-	+	+	-
	B3	+	-	+	-	+
BPA	B1	-	-	+	+	-
	B2	+	-	-	+	+
TSA	B1	+	-	+	+	-
	B2	+	-	+	+	+
	B3	+	-	+	+	-

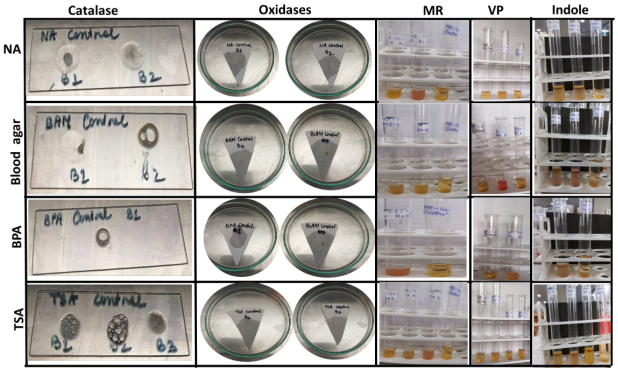

Fig. 8. Biochemicals test results of bacterial strains from soil sample on different media's (A - NA, B - Blood Agar, C - Beef Peptone Agar, D – TSA)

Table 6. Biochemical Test Results for Bacterial Strains Isolated from Soil Sample on Different Media

Media	Colony Name	Catalase Test	Oxidase Test	Indole Test	MR	VP
NA	B1	+	-	-	-	-
	B2	+	-	-	+	-
BAM	B1	-	-	-	-	-
	B2	+	-	-	-	+
BPA	B1	+	-	-	+	-
TSA	B1	+	-	-	-	-
	B2	+	-	-	-	-
	B3	+	-	-	+	-

Identifying Gut Microbial Diversity through Biochemical Testing in Captive Tigers: In this study, the results obtained from biochemical tests and Gram staining were further analyzed through the ABIS software to identify potential bacterial species based on their biochemical profiles. Through this approach, various bacterial strains were successfully identified across different media types at respective dilutions of 10^{-5} for male tiger and soil samples, and 10^{-3} for the female tiger sample which is given below in Table 7, 8 and 9.

Table 7. Biochemical Test Results and Gram Staining of Bacterial Colonies from Male Tiger Fecal Sample on Different Media with Possible Bacteria Identification.

S. No.	Type of Media	Bacterial Colony	Gram Staining Result	Biochemical Tests					Characteristics/ Possible Bacteria
				Catalase	Oxidase	Indole	MR	VP	
1	NA	B1	+	+	-	+	+	-	*Staphylococcus aureus*
		B2	-	+	-	-	+	-	*Escherichia coli*
		B3	-	+	-	+	-	-	*Salmonella spp.*
		B4	+	-	-	+	+	-	*Bacillus subtilis*
2	MAC	B1	-	+	-	+	+	-	*Pseudomonas aeruginosa*
		B2	-	+	-	+	+	-	*Klebsiella pneumoniae*
		B3	-	+	-	+	+	-	*Proteus mirabilis*
		B4	-	+	-	+	+	-	*Citrobacter freundii*
		B5	+	+	-	+	+	-	*Enterococcus faecalis*
3	BAM	B1	+	+	-	-	-	-	*Staphylococcus epidermidis*
		B2	-	+	-	+	+	+	*Klebsiella oxytoca*
		B3	-	+	-	+	+	-	*Shigella flexneri*
		B4	+	+	-	+	+	-	*Bacillus cereus*
4	BPA	B1	+	+	-	+	+	-	*Streptococcus pyogenes*
		B2	-	-	-	+	+	-	*Streptococcus agalactiae*
		B3	-	-	-	+	-	-	*Lactobacillus spp.*
5	TSA	B1	+	+	-	+	+	-	*Micrococcus luteus*
		B2	-	+	-	-	+	-	*Escherichia coli*
		B3	-	+	-	+	+	-	*Enterobacter cloacae*

Table 8. Biochemical Test Results and Gram Staining of Bacterial Colonies from Female Tiger Fecal Sample on Different Media with Possible Bacteria Identification

S.No.	Type of Media	Bacterial Colony	Gram Staining Result	Biochemical Tests					Characteristics/ Possible Bacteria
				Catalase	Oxidase	Indole	MR	VP	
1	NA	B1	+	+	-	+	+	-	*Staphylococcus aureus*
		B2	-	+	-	+	+	+	*Enterococcus faecalis*
2	MAC	B1	-	+	-	+	+	-	*Escherichia coli*
		B2	-	+	-	+	+	-	*Klebsiella pneumoniae*
3	BAM	B1	-	+	-	-	+	-	*Pseudomonas aeruginosa*
		B2	-	+	-	+	+	-	*Bacillus subtilis*
		B3	+	+	-	+	-	+	*Enterobacter cloacae*
4	BPA	B1	-	-	-	+	+	-	*Enterococcus faecalis*
		B2	+	+	-	-	+	+	*Bacillus cereus*
5	TSA	B1	+	+	-	+	+	_	*Bacillus cereus*
		B2	-	+	-	+	+	+	*Bacillus cereus*
		B3	-	+	-	+	+	-	*Klebsiella pneumoniae*

Table 9. Biochemical Test Results and Gram Staining of Bacterial Colonies from Soil Sample on Different Media with Possible Bacteria Identification

S.No.	Type of Media	Bacterial Colony	Gram Staining Result	Biochemical Tests					Characteristics/ Possible Bacteria
				Catalase	Oxidase	Indole	MR	VP	
1	NA	B1	-	+	-	-	-	-	*Enterococcus faecalis*
		B2	-	+	-	-	+	-	*Klebsiella pneumoniae*
2	BAM	B1	+	-	-	-	-	-	*Bacillus cereus*
		B2	+	+	-	-	-	+	*Pseudomonas aeruginosa*
3	BPA	B1	-	+	-	-	+	-	*Klebsiella pneumoniae*
4	TSA	B1	+	+	-	-	-	-	*Staphylococcus aureus*
		B2	-	+	-	-	-	-	*Bacillus cereus*
		B3	-	+	-	-	+	-	*Escherichia coli*

Six bacteria (*Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Bacillus cereus*) were found in all three sample types. Several bacteria were shared between the male and female tiger fecal samples and between the female tiger and soil or the male tiger and soil. Unique bacteria found in the male tiger fecal sample include *Salmonella spp.*, *Proteus mirabilis,Micrococcus luteus,Citrobacter freundii, Staphylococcus epidermidis* and *Klebsiella oxytoca.* The female tiger fecal sample contained *Bacillus subtilis* and *Enterobacter cloacae* as unique findings. No bacteria were found to be exclusive to the soil sample.

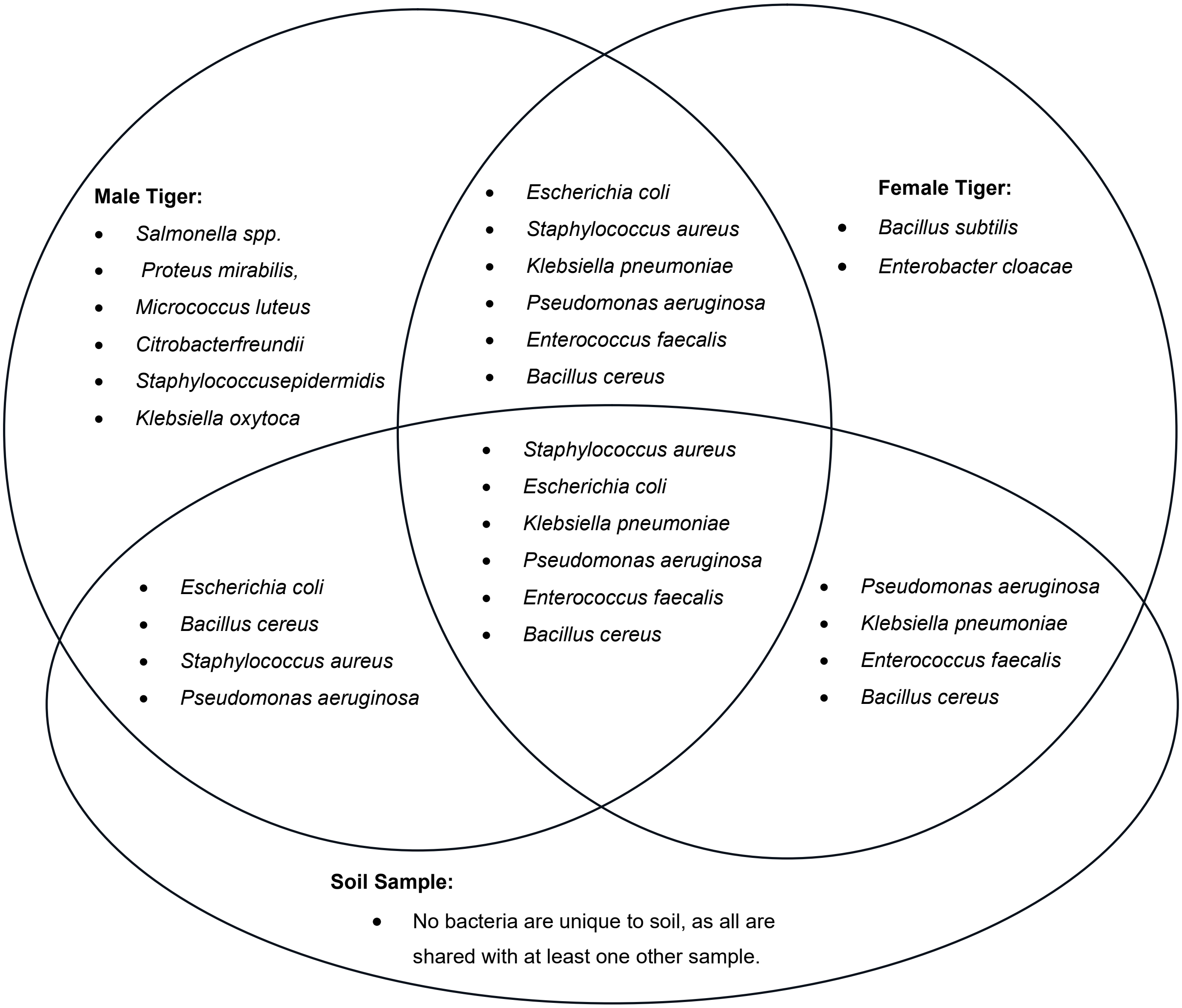

Fig. 9. Venn diagram illustrating the bacterial species found in male tiger fecal sample, female tiger fecal sample and soil sample

Metabolic and Microbial Diversity Analysis of Bacterial Species in Tiger Gut Microbiome: Table 10 outlines the metabolic characteristics and possible pathogenic effects of various bacterial species present in the gut microbiome of captive tigers. The bacterial diversity observed includes members from the *Firmicutes*, *Proteobacteria* and *Actinobacteria* phyla, each contributing distinct metabolic functions and occasionally associated with gastrointestinal or systemic infections.

Table 10. Metabolic and Microbial Diversity Analysis of Bacterial Species in Tiger Gut Microbiome

S.No.	Bacteria	Phylum	Associated Diseases
1	*Staphylococcus aureus*	*Firmicutes*	Skin Infections, Pneumonia.
2	*Bacillus subtilis*	*Firmicutes*	Gastroenteritis, Food Poisoning.
3	*Enterococcus faecalis*	*Firmicutes*	Urinary Tract Infections.
4	*Staphylococcus epidermidis*	*Firmicutes*	Diarrhea, bloating.
5	*Bacillus cereus*	*Firmicutes*	Food Poisoning.
6	*Escherichia coli*	*Proteobacteria*	Gastroenteritis, Urinary Tract Infections.
7	*Salmonella spp.*	*Proteobacteria*	Salmonellosis.
8	*Pseudomonas aeruginosa*	*Proteobacteria*	Respiratory Infections.
9	*Klebsiella pneumoniae*	*Proteobacteria*	Pneumonia, Urinary Tract Infections.
10	*Proteus mirabilis*	*Proteobacteria*	Urinary Tract Infections.
11	*Citrobacter freundii*	*Proteobacteria*	Urinary Tract Infections.
12	*Klebsiella oxytoca*	*Proteobacteria*	Respiratory Infections.
13	*Shigella flexneri*	*Proteobacteria*	Shigellosis.
14	*Streptococcuspyogenes*	*Proteobacteria*	Gastrointestinal Issues.
15	*Streptococcus agalactiae*	*Proteobacteria*	Neonatal Infections.
16	*Lactobacillus spp.*	*Proteobacteria*	Gastrointestinal Disorders.
17	*Enterobacter cloacae*	*Proteobacteria*	Diarrhea, Urinary Tract Infections.
18	*Micrococcus luteus*	*Actinobacteria*	Respiratory Infections in Captivity.

The analysis reveals that these bacteria play crucial roles in essential metabolic processes, such as digesting complex carbohydrates, synthesizing amino acids and producing short-chain fatty acids. This microbial diversity is vital for maintaining gut health and efficient nutrient absorption, underscoring the complex dietary needs of these apex predators.

Discussion:

This study aimed to analyze the microbial communities in the fecal samples of Bengal tigers (male and female) and the surrounding soil at Patna Zoo. The results highlighted differences in microbial diversity between the fecal samples and the soil, with the feces showing a broader range of bacterial species, while the soil samples had fewer bacterial populations. This reflects the distinct microbial environments of the tiger's gut versus its habitat. The biochemical tests performed on the isolated bacterial species provided insights into their metabolic characteristics, suggesting a variety of functional roles in the tiger's gut. These findings indicate that the gut microbiota is diverse and plays a significant role in the digestion and health of the tigers. Comparing the microbial communities of zoo tigers with wild tigers from available data suggests that captivity may alter the microbial diversity of zoo tigers, potentially due to differences in diet, healthcare and environmental exposure. The pH of the fecal samples also provides insight into the digestive conditions which could influence microbial growth.

Overall, this study contributes to understanding the microbial populations in captive Bengal tigers and their environment, laying the foundation for future research on how captivity impacts gut health and microbial diversity in wild and captive species. Further studies could explore the long-term effects of captivity on tiger microbiota and its implications for animal welfare.

Conclusion:

Differences in the gut microbiota between captive and wild tigers are primarily influenced by their diet and living environments. Therefore, we propose that aligning the gut microbiome of captive tigers with that of wild tigers should be a key focus in preparation for reintroduction, serving as a critical criterion for evaluating whether reintroduction efforts should proceed. In this regard, we recommend the following strategies for reintroduction preparation:

Rewilding efforts should not only assess an animal's ability to hunt but also its adaptability to wild food sources and environmental conditions. The rewilding site should closely mimic the tiger's natural habitat, and over time, their diet should be progressively shifted to include natural prey species found in the wild. Furthermore, human and medical interventions should be minimized. Ideally, the rewilding area should expand gradually, encompassing a variety of wild prey species and habitat types, thus facilitating the establishment of a gut microbiota more suited to the wild environment. The duration of rewilding training must be extended to ensure a successful transition of the gut microbiota from its captive to wild structure. A comprehensive monitoring database on the gut microbiota of wild tigers should be created to serve as a baseline for comparison. Once significant convergence in the composition and function of the gut microbiota between captive and wild tigers is achieved, the chances of successful reintroduction are likely to improve.

Further research is essential to better understand the complex interactions between host genetics, environmental factors, dietary shifts, and the gut microbiota of Bengal tigers. Additionally, employing advanced techniques such as transcriptomics to explore the functional annotation of genes and host traits will provide deeper insights into how the physiology and immunology of tigers may influence the success of their reintroduction.

References :

Amato, K. R., Leigh, S. R., Kent, A., Mackie, R. I., Yeoman, C. J., Stumpf, R. M., ... & Garber, P. A. (2015). The gut microbiota appears to compensate for seasonal diet variation in the wild black howler monkey (Alouatta pigra). Microbial ecology, 69, 434-443.

Amato, K. R., Yeoman, C. J., Kent, A., Righini, N., Carbonero, F., Estrada, A., ... & Leigh, S. R. (2013). Habitat degradation impacts black howler monkey (Alouatta pigra) gastrointestinal microbiomes. The ISME journal, 7(7), 1344-1353.

Backhed, F., Ley, R. E., Sonnenburg, J. L., Peterson, D. A., & Gordon, J. I. (2005). Host-bacterial mutualism in the human intestine. science, 307(5717), 1915-1920.

Barlow, A. C., Mazák, J., Ahmad, I. U., & Smith, J. L. (2010). A preliminary investigation of Sundarbans tiger morphology.

Becker, A. A., Hesta, M., Hollants, J., Janssens, G. P., & Huys, G. (2014). Phylogenetic analysis of faecal microbiota from captive cheetahs reveals underrepresentation of Bacteroidetes and Bifidobacteriaceae. BMC microbiology, 14, 1-11.

Clayton, J. B., Vangay, P., Huang, H. U., Ward, T., Hillmann, B. M., Al-Ghalith, G. A., ... & Knights, D. (2016). Captivity humanizes the primate microbiome. Proceedings of the National Academy of Sciences, 113(37), 10376-10381.

Clemente, J. C., Ursell, L. K., Parfrey, L. W., & Knight, R. (2012). The impact of the gut microbiota on human health: an integrative view. *Cell*, *148*(6), 1258-1270.

Cresci, G. A., & Bawden, E. (2015). Gut microbiome: what we do and don't know. Nutrition in Clinical Practice, 30(6), 734-746.

David, L. A., Maurice, C. F., Carmody, R. N., Gootenberg, D. B., Button, J. E., Wolfe, B. E., ... & Turnbaugh, P. J. (2014). Diet rapidly and reproducibly alters the human gut microbiome. Nature, 505(7484), 559-563.

Degnan, P. H., Pusey, A. E., Lonsdorf, E. V., Goodall, J., Wroblewski, E. E., Wilson, M. L., ... & Ochman, H. (2012). Factors associated with the diversification of the gut microbial communities within chimpanzees from Gombe National Park. Proceedings of the National Academy of Sciences, 109(32), 13034-13039.

Delport, T. C., Power, M. L., Harcourt, R. G., Webster, K. N., & Tetu, S. G. (2016). Colony location and captivity influence the gut microbial community composition of the Australian sea lion (Neophoca cinerea). Applied and Environmental Microbiology, 82(12), 3440-3449.

Ding, T., & Schloss, P. D. (2014). Dynamics and associations of microbial community types across the human body. Nature, 509(7500), 357-360

Gani, M., Mohd-Ridwan, A. R., Sitam, F. T., Kamarudin, Z., Selamat, S. S., Awang, N. M. Z., ... & Md-Zain, B. M. (2024). Habitat shapes the gut microbiome diversity of Malayan tigers (Panthera tigris jacksoni) as revealed through metabarcoding 16S rRNA profiling. *World Journal of Microbiology and Biotechnology*, *40*(4), 111.

Gilbert, M., Sulikhan, N., Uphyrkina, O., Goncharuk, M., Kerley, L., Castro, E. H., ... & Cleaveland, S. (2020). Distemper, extinction, and vaccination of the Amur tiger. Proceedings of the National Academy of Sciences, 117(50), 31954-31962.

Goodrich, J., Lynam, A., Miquelle, D., Wibisono, H., Kawanishi, K., Pattanavibool, A., ... & Karanth, U. (2015). Panthera tigris. The IUCN Red List of Threatened Species 2015: e. T15955A50659951.

He, F., Liu, D., Zhang, L., Zhai, J., Ma, Y., Xu, Y., ... & Ma, J. (2018). Metagenomic analysis of captive Amur tiger faecal microbiome. *BMC Veterinary Research*, *14*, 1-8.

Hooper, L. V., Littman, D. R., & Macpherson, A. J. (2012). Interactions between the microbiota and the immune system. science, 336(6086), 1268-1273.

Hyde, E. R., Navas-Molina, J. A., Song, S. J., Kueneman, J. G., Ackermann, G., Cardona, C., ... & Knight, R. (2016). The oral and skin microbiomes of captive komodo dragons are significantly shared with their habitat. Msystems, 1(4), 10-1128.

Jackson, P. (1999). The tiger in human consciousness and its significance in crafting solutions for tiger conservation. Riding the tiger: Tiger conservation in human-dominated: landscapes. Cambridge University Press, Cambridge, 50-60.

Jhala, Y. V., Qureshi, Q., & Nayak, A. K. (Eds.). (2021). *Status of Tigers, Co-predators and Prey in India 2018*. National Tiger Conservation Authority, New Delhi and Wildlife Institute of India, Dehradun.

Khairulmunir, M., Gani, M., Karuppannan, K. V., & Md-Zain, B. M. (2023). High-throughput DNA metabarcoding for determining the gut microbiome of captive critically endangered Malayan tiger (Pantheratigrisjacksoni) during fasting. *Biodiversity Data Journal*, *11*.

Ley, R. E., Hamady, M., Lozupone, C., Turnbaugh, P. J., Ramey, R. R., Bircher, J. S., ... & Gordon, J. I. (2008). Evolution of mammals and their gut microbes. science, 320(5883), 1647-1651.

Ley, R. E., Peterson, D. A., & Gordon, J. I. (2006). Ecological and evolutionary forces shaping microbial diversity in the human intestine. Cell, 124(4), 837-848.

Ley, R. E., Turnbaugh, P. J., Klein, S., & Gordon, J. I. (2006). Human gut microbes associated with obesity. nature, 444(7122), 1022-1023.

Liu, E., Ma, L., You, D., Yang, C., Hu, Y., Xu, H., ... & Wang, Y. (2021). Haematological and biochemical parameters of captive siberian tigers (Panthera tigrisaltaica) from the Heilongjiang Province, China. Veterinary Medicine and Science, 7(3), 1015-1022.

Luo, S. J., Kim, J. H., Johnson, W. E., Walt, J. V. D., Martenson, J., Yuhki, N., ... & O'Brien, S. J. (2004). Phylogeography and genetic ancestry of tigers (Panthera tigris). PLoS biology, 2(12), e442.

McKenzie, V. J., Song, S. J., Delsuc, F., Prest, T. L., Oliverio, A. M., Korpita, T. M., ... & Knight, R. (2017). The effects of captivity on the mammalian gut microbiome. Integrative and comparative biology, 57(4), 690-704.

Muegge, B. D., Kuczynski, J., Knights, D., Clemente, J. C., González, A., Fontana, L., ... & Gordon, J. I. (2011). Diet drives convergence in gut microbiome functions across mammalian phylogeny and within humans. Science, 332(6032), 970-974.

Nelson, K. E., & colleagues. (2015). The human microbiome project: A comprehensive analysis of the gut microbiome. *Nature Reviews Microbiology*, 13(5), 326-338.Nicholson, J. K., Holmes, E., Kinross, J., Burcelin, R., Gibson, G., Jia, W., & Pettersson, S. (2012).

Ozburn, R. H. (1940) “Bio-ecology. By F. E. Clement and V. E. Shelford. John Wiley & Sons, Inc., New York. Pages i—vi and 1—425; 85 figures. 1939. Price $4.50.,” The Canadian Entomologist, Cambridge University Press, 72(5), pp. 108–108.

Patna Zoo Official Website. (2023). Sanjay Gandhi Biological Park.

Schmidt, T. S., Raes, J., & Bork, P. (2018). The human gut microbiome: from association to modulation. Cell, 172(6), 1198-1215.

Seidensticker, J., & McDougal, C. (1993). Tiger predatory behaviour, ecology and conservation. In Symposium of the zoological society of London.

Sommer, F., & Bäckhed, F. (2013). The gut microbiota—masters of host development and physiology. Nature reviews microbiology, 11(4), 227-238.

Sunquist, F. (2002). Wild Cats of the World. The University of Chicago Press.

Sunquist, M. (1999). Ecology, behaviour and resilience of the tiger and its conservation needs. Riding the tiger: tiger conservation in human dominated landscapes, 5-18.

Sunquist, M. E. (1981). The social organization of tigers (Panthera tigris) in Royal Chitawan National Park, Nepal.

Tung, J., Barreiro, L. B., Burns, M. B., Grenier, J. C., Lynch, J., Grieneisen, L. E., ... & Archie, E. A. (2015). Social networks predict gut microbiome composition in wild baboons. elife, 4, e05224.

Turnbaugh, P. J., Ley, R. E., Mahowald, M. A., Magrini, V., Mardis, E. R., & Gordon, J. I. (2006). An obesity-associated gut microbiome with increased capacity for energy harvest. nature, 444(7122), 1027-1031.

Weber, W., & Rabinowitz, A. (1996). A global perspective on large carnivore conservation. Conservation biology, 10(4), 1046-1054.

World Animal Foundation. (2007). Tiger Fact Sheet. Retrieved from World Animal Foundation.

WWF-INDIA (2014) All about tigers. Retrieved from http://www.wwfindia.org/about_wwf/priority_species/royal_bengal_tiger/

Zhang, X., Liao, Y., Qin, T., Ma, J., Liu, J., Zou, J., ... & Yang, M. (2022). Developmental stage variation in the gut microbiome of South China tigers. *Frontiers in Microbiology*, *13*, 962614.

Zoetendal, E. G., Akkermans, A. D., Akkermans-van Vliet, W. M., de Visser, J. A. G., & de Vos, W. M. (2001). The host genotype affects the bacterial community in the human gastronintestinal tract. *Microbial ecology in health and disease*, *13*(3), 129-134.

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