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ORIGINAL ARTICLE

Metagenomic analysis of intestinal microbiota in wild rats living in urban and rural habitats

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Abstract

Mammals have a symbiotic relationship with various microorganisms called microbiota throughout their lives. These microorganisms are known to affect the host's physiology, health, and even mental balance. The development of the gut microbiota is regulated by a complex interaction between host and environmental factors, including diet and lifestyle. Herein, it is aimed to elucidate the differences in the gut microbiota of rats living in urban and rural habitats. The taxonomic changes in the gut microbiota of wild rats belonging to *Rattus rattus* species caught from urban and rural areas of Western Anatolian (Bilecik province) were examined comparatively by 16S rRNA next-generation sequencing technique. Laboratory rats were used as reference animals. The alpha diversities were found lower in the rural rats with respect to the urban rats, whereas the highest alpha diversity was calculated for laboratory rats. The lower Firmicutes to Bacteroidetes ratios (F/B ratio) were accounted for both rural and laboratory rats compared with urban rats. The Proteobacteria to Actinobacteria ratio (P/A ratio) was lower for rural rats, but higher for laboratory rats, compared with urban rats. The heatmap analyses of taxonomic units in the microbiota of each group demonstrated distinct patterns at the species and genus levels. The study provided metagenomic data on the gut microbiota of rats residing in urban and rural habitats, offering a different perspective on future environmental biomonitoring studies.

Introduction

Microbiota forms depending on the interaction with the environment after birth (Wopereis et al. 2014). Nutrition, genetic factors, age, geography, and even the way of birth affect the maturation process of the microbiota (Flandroy et al. 2018). Mammalian microbiota has various compositions in different animal species and is quite complex and widescale depending on their anatomical features and nutritional preferences (omnivorous, carnivore, herbivore) (Flemer et al. 2017). A total of 8 bacterial phyla have been mainly identified in the human gut microbiota as Firmicutes, Bacteroidetes, Actinobacteria, Fusobacteria, Proteobacteria, Verrucomicrobia, Cyanobacteria, and Spirochaetes. The human and mouse/rat microbiota are similar in terms of microbial diversity, except for Fusobacteria (Bäckhed et al. 2005). According to the microbial sequence data of humans and rats, Firmicutes and Bacteroidetes (93%) were similarly dominant in both (Lleal et al. 2019). In other studies, it has been confirmed that the gut microbiota of laboratory rats is more similar to humans than mice (Manichanh et al. 2010).

The development of the gut microbiota is regulated by a complex interaction between the host and numerous factors, including diet and lifestyle (Rothschild et al. 2018). The dynamics of the gut microbiota from birth to adult age can shed light on the variability of this community within the host and its relationships with disease risks (Bäckhed et al. 2015). Foods such as snails, arthropods, mushrooms, seeds, leaves, fruits, flowers, bark, and stems constitute the natural diet of rats. Various seeds and leaves are detected in the stomach content of *Rattus rattus* living in steppe areas as 70% fruits, 15% arthropods, 8% seeds, 2% root, 1% flower, 1% grass leaf, and ≤ 1% trace nutrients. However, since rats can adapt to a wide variety of foods, their diet preference is largely determined by aliment availability. For rats that cannot find natural sources in urban life, rotten food found in the litter is a primary food source (Traweger and Slotta-Bachmayr 2005).

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Studies conducted by taking into consideration the pollutant concentrations in plant cover and invertebrates, which are the primary food sources of rodents, have provided important information about environmental pollution. Therefore, in terrestrial habitats, rats and mice are frequently used as biomonitors to reveal environmental pollution caused by heavy metals, inorganic pollutants, radioactive pollutants, and organic pollutants (Jankovská et al. 2009). In the vast majority of microbial studies, the effect of contaminants on gut microbiota was determined by analyzing the stool microbiome with high throughput sequencing techniques (Breton et al. 2013). The effects of pollutants such as heavy metals and pesticides on the different rodent microbiota were investigated in these studies carried out in a laboratory environment (Fang et al. 2018). Recent studies that reveal the surprising effects of intestinal microbiota on human psychology and metabolism have been commonly utilized in vivo rodent models (rats and mice) in addition to other model organisms (Licht and Bahl 2019). One of these studies demonstrated that the application of antibiotic drugs at juvenile age seriously affects the behavior, cognition, and memory of mice at adolescence age in line with the modulations in gut microbiota (Ceylani et al. 2018). Furthermore, the impact of intestinal microbiological changes on numerous human diseases has recently made microbiota researches the center of attention by the public and scientific community (Helmink et al. 2019). Because of the similarities in gut microbiota composition between humans and rodents, these models are an important tool in evaluating the harmful effects of all microbiota modifying factors.

Dietary preferences are recognized as a primary determinant of gut microbiota composition in mammals, militating even genetic ones (Carmody et al. 2019). Today, the scientific community is also aware of the fact that the living environment/habitat can also affect the gut microbiota; however, to the best of our knowledge, these in vitro and in vivo data were mainly obtained using laboratory models, animals, and/or humans. However, the animals living in their natural habitats as a part of our ecosystem can introduce a piece of valuable biological information about our lifestyle and health, and also serve as bioindicators of environmental pollution levels in the air, water, and soil. This study aimed to understand how urban and rural habitats modulate the gut microbiota of natural rat populations. To this end, a metagenomic approach that is 16S rRNA next-generation sequencing (NGS) of bacterial populations was employed to compare the gut microbiota patterns between rats caught from urban and rural environments. The attention was given to the elaboration of unique bacterial species and genera. Moreover, the indices of alpha diversity (Shannon and Simpson values) and healthy gut homeostasis (Firmicutes to Bacteroidetes ratio -F/B ratio) were calculated. Since the Proteobacteria (P) and Actinobacteria (A) are among the most abundant phyla in gut microbiota and implicated in various human diseases, the P/A ratio was also evaluated. The study is valuable as it provides microbiota information on the of urban and rural life on mammals which can shed light on studies dealing with the influence of urban and rural lifestyles on human health. Furthermore, the provided gut microbiota data may offer a different perspective on future environmental biomonitoring studies.

Materials and methods

Animals and sampling

Rattus rattus samples were divided into two different groups according to their habitats in Western Anatolia (Bilecik province). The urban habitat group (RH1) consists of the wild rat samples collected from the industrial area, whereas the rural habitat group (RH2) comprises the wild rats living in cow farms (Fig. 1). The distance between these habitats was estimated to be approximately 11 km. Rats were collected using 50 live traps that were randomly left to the studied area (≈1000 m^2). The animals were collected with permission (permission number: 21264211–288.04-E.3311026) of the General Directorate of Nature Conservation and National Parks affiliated to the Turkish Ministry of Agriculture and Forestry. Laboratory rats (RL) group were kindly provided by Aydın Adnan Menderes University (project number: TPF17040). Only male rats were included in the study to prevent sexual variations in the microbiota. The animals collected from the urban and rural habitats during June 2019 were brought to the laboratory on the day of capture, and ether inhalation anesthesia was used after a fasting period of 4 h. The same procedure was applied to laboratory rats. After this step, the cecum (a pouch that connects the small intestine to the colon) part of the proximal colon was dissected immediately; its contents were taken with sterile swabs, frozen with liquid nitrogen, and stored in sterile tubes at −80 °C until the next use. Only sterile-single use and pre-sterilized instruments were used throughout the process.

DNA isolation

For each experimental group, three (3) pools of feces obtained from the cecum of different animals were subjected to DNA isolation. Each pool was created by combining feces obtained from 6 to 7 animals. Input DNA was isolated by Quick-DNA TM Fecal/Soil Microbe Miniprep Kit (catalog number: D6010/Zymo Research, USA) according to the manufacturer's instructions. The amount and purity of the isolated DNA were determined fluorometrically with Qubit Fluorometer (Invitrogen, USA). For optimum performance,

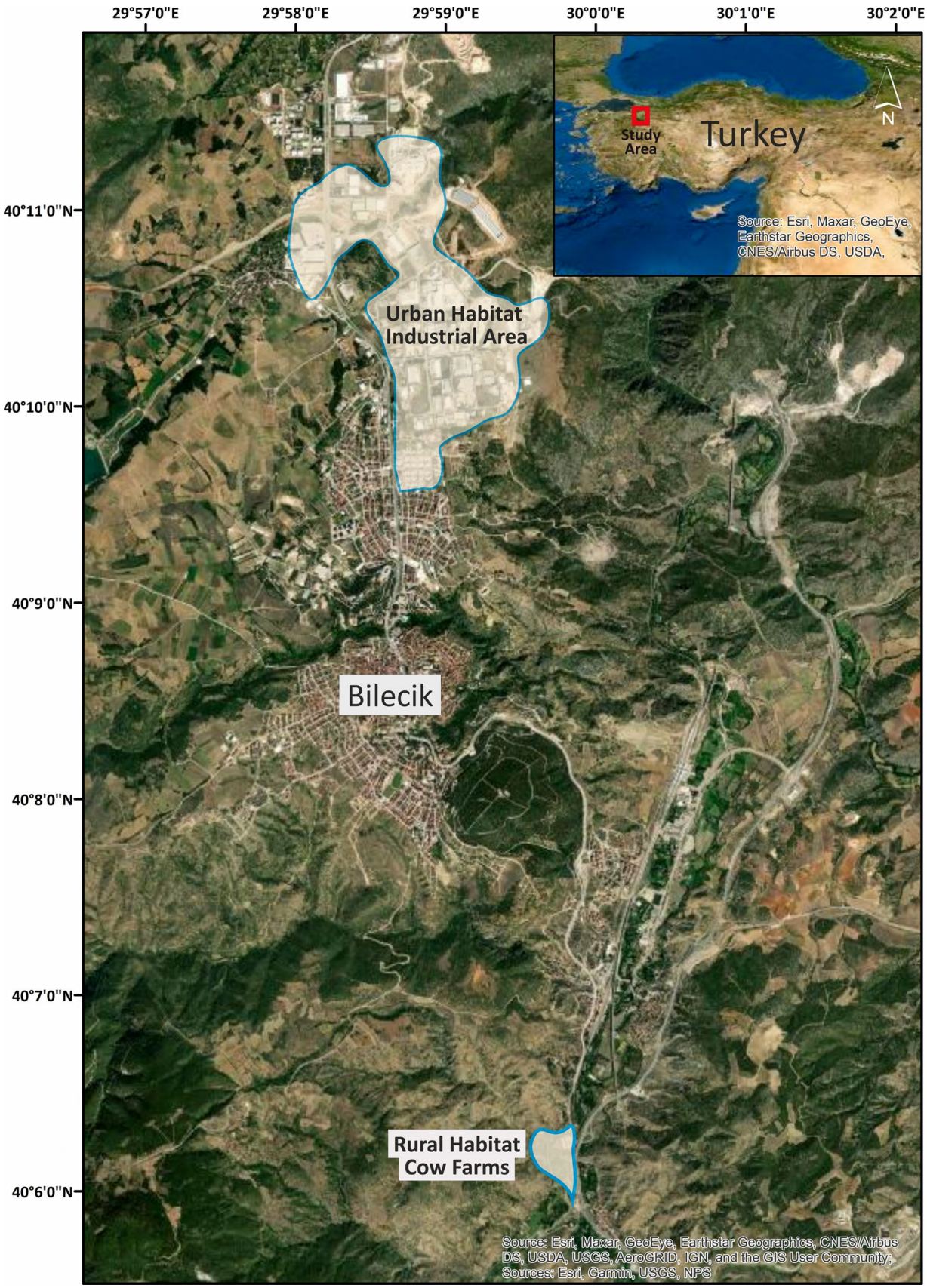

Fig. 1 Satellite image of the locations where the wild rats were collected

0.5% (v/v) β-mercaptoethanol was added to the final dilution of the genomic lysis buffer.

Amplification of V3–V4 hypervariable region of 16S rRNA gene

DNA load before the PCR step is equalized between samples. The V3–V4 regions of the 16S rRNA gene to be used in species determination were amplified with the 341F (CCT ACGGGNGGCWGCAG)-805R (GACTACHVGGGTAT CTAATCC) primer sequences using the polymerase chain reaction method in a thermal cycler (Applied Biosystems SimpliAmp Thermal Cycler Thermo Fisher Scientific, USA) (Shahi et al. 2019; Sune et al. 2020; Drengenes et al. 2021). The reaction mixture ratios were prepared as 25 µl for each sample (Table 1). PCR was completed by reducing the temperature to 4 °C.

NGS library preparation and sequencing

Before the sequencing, 16S rRNA V3-V4 amplicon products were purified by Column -Pure PCR Clean-Up Kit (catalog number: D509 /ABMGood, CAN). Library preparation for 16S rRNA V3-V4 amplicon products was accomplished using Nextera XT DNA Library Prep Kit (catalog number: FC-131–1096, Illumina, USA). Index operation was done by TG Nextera XT Index Kit v2 Set A /96 Indices, 384 Samples (catalog number: TG-131–2001, Illumina, USA). Sequencing was performed with the Illumina Miseq platform (Illumina, USA) as paired-end (PE) 2 × 150.

Table 1 Polymerase chain reaction components and their quantities (Shahi et al. 2019; Sune et al. 2020; Drengenes et al. 2021)

PCR components	Concentration (µl)
Master mix	12.5 µl
Forward primer	0.5 µl
Reverse primer	0.5 µl
dH_20	9.5 µl
Genomic DNA	2.0 µl
PCR reactions were carried out in a total volume of 25 µl containing	
Thermal profile	**Time**
İnitial denaturation at 95 °C	10 min
Followed as 35 cycles of	
Denaturation at 95 °C	30 s
Annealing at 50–55 °C	30 s
Extension at 72 °C	30 s

Bioinformatic analysis of raw data

Raw data readings (FASTQ) were made by separating the metadata into the operational taxonomic unit (OTU) classes with the Kraken Metagenomic system. Kraken system assigns taxonomic tags to short DNA sequences with high sensitivity and speed (Wood and Salzberg 2014). Taxonomic assignment was done by using a commercially available OmicsBox Metagenomics module, which uses NCBI's taxonomy, Greengenes, SILVA, and RDP databases. From quality check to the OTU assignment, all workflow was conducted with this tool. For quality control (using FastQC software); adaptor, contaminant, and low-quality sequences (< Q20) were trimmed. Bacterial distribution and diversity in the samples are presented as heatmap patterns and bar graphs, respectively.

Statistics

The statistical analyses were given as mean ± standard deviation (SD). Tukey's multiple comparisons test in prdinary one-way ANOVA was utilized to compare the alpha diversities and F/B ratio between the experimental groups. The comparison was done in GraphPad Prism 8 (GraphPad Software, USA) software. The degree of significance was denoted as $*p < 0.05$, $***p < 0.001$, and $****p < 0.0001$. Heatmap analysis of metagenomic counts for bacterial genera and species was performed in GraphPad Prism 8 (GraphPad Software, USA) software.

Results and discussion

Bacterial diversity in gut microbiota

To determine the gut microbiota changes in urban (RH1), rural (RH2), and laboratory rats (RL), the bacterial alpha diversity indices were calculated (Fig. 2). The Shannon and Simpson indices reflect the alpha diversity in any population (Zhang et al. 2017). In terms of microbial ecology, analyzing the alpha diversity of amplicon sequencing data is known to be the common first step in assessing differences between microbial environments. Shannon and Simpson indices are both popular parameters to assess microbial richness and evenness. The former increases with richness and evenness, and it puts more weight on the richness than on evenness, whereas the Simpson index is more influenced by evenness than richness (Matthews 2014). The results have shown that the RL group has the highest alpha diversity compared with both RH1 and RH2 groups, whereas the diversities were found lower in the RH2 group with respect to the RH1 group (Fig. 2a, b). Therefore, our results can be interpreted as a larger bacterial richness (Shannon index) and evenness

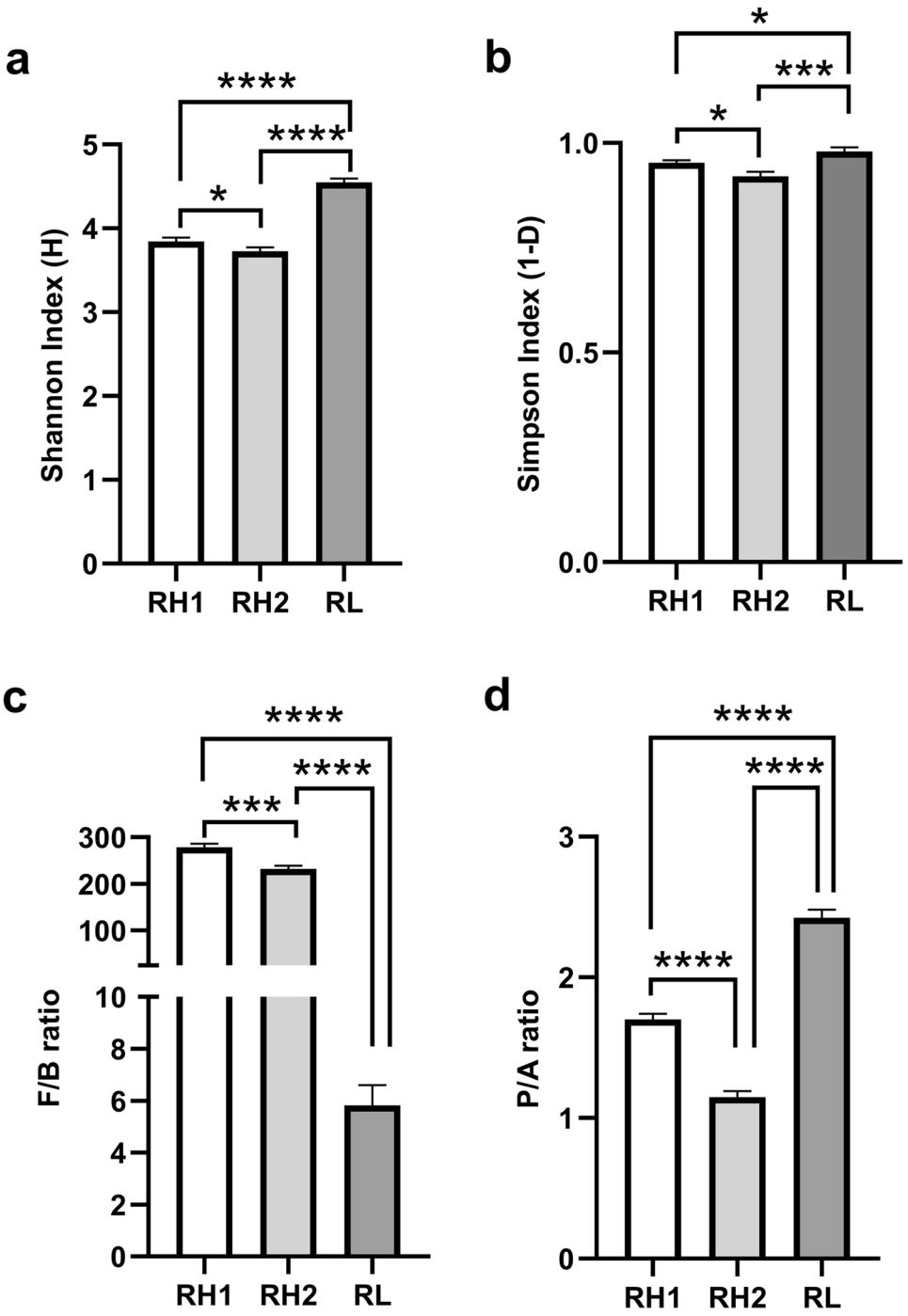

Fig. 2 **a** Shannon index (H), **b** Simpson index (1-D), **c** Firmicutes to Bacteroidetes ratio (F/B ratio), and **d** Proteobacteria to Actinobacteria ratio (P/A ratio) in the microbiota. RH1: Rat samples from the urban habitat, RH2: Rat samples from the rural habitat, RL: Laboratory rat samples

(Simpson index) in the RH1 group. In other words, the number of different species in the microbiota of urban rats was found larger than that of the rural ones. These findings are logical since the urban environment harbors a greater variety of food choices than the rural one.

The lower F/B ratios were accounted for both RH2 and RL groups with respect to RH1 (Fig. 2c). Firmicutes and Bacteroidetes are the two most common bacterial phyla in the human microbiome; therefore, perturbations in the proportional composition of these two taxonomical groups may provide insight into host health status (Razavi et al. 2019). The F/B ratio is a widely used biomarker in microbiota studies to propose a relationship of microbiota with health status and/or different pathological conditions (Magne et al. 2020). It rises from birth to adulthood, and high F/B ratios are associated with a dysbiotic microbiome (Indiani et al. 2018). The P/A ratio was lower for the RH2 group, but higher for the RL group, compared with RH1 (Fig. 2d).

Proteobacteria are reported to be high in several gut-related and non-gut disorders, predominantly with inflammatory traits; therefore, this phylum is suggested as a "microbial signature" of the disease state (Rizzatti et al. 2017). On the other hand, Actinobacteria are fundamental players in maintaining gut barrier homeostasis, and species belonging to this phylum are universally utilized as probiotic supplements, which are valuable in the prevention of many diseases (Binda et al. 2018).

Taxonomic distributions of bacterial genera in gut microbiota

The distribution of dominant bacterial genera in the microbiota is also compared between study groups considering metagenomic counts (Fig. 3). According to heatmap patterns, each group has unique genera composition. *Lactobacillus*, *Lachnoclostridium*, *Mordavella*, *Pedicoccus*, *Streptococcus*, *Desulfovibrio*, *Anaerostipes*, *Blautia*, *Clostridium*, and *Escherichia* are the main genera in descending order for the RH1 group. *Pediococcus* genus was unique for the RH1 group. On the other hand, *Herbinix* and *Roseburia* genera were specific for the RH2 microbiota, in which the *Pedicoccus* and *Clostridium* were absent. The genus profile in the RL group contains unique players such as *Duncaniella*, *Ruminococcus*, *Flavonifractor*, *Muribaculum*, *Intestinimonas*, *Candidatus*, and *Marinilactibacillus*. The bacterial genera were evenly distributed in the microbiota of laboratory rats with slight deviations. However, two different genera (*Lactobacillus* and *Lachnoclostridium*) comprise much of the bacterial population in wildlife specimens. This situation may explain the observed low alpha diversities in wildlife groups compared with laboratory rats. In other words, the presence of a predominant species in wild rats' microbiota may have prevented other bacterial species from participating in microbiota activities.

Taxonomic distributions of bacterial species in gut microbiota

The dominant bacterial species of the microbiota are presented as heatmap visualization across studied groups considering metagenomic counts (Fig. 4). Accordingly, the microbiota of each group shows distinct profiles at the species level. *Lactobacillus reuteri* was found to be the most common type of bacteria in both RH1 and RH2 groups, which was absent in the RL group microbiota. Similarly, [*Clostridium*] *scindens*, *Mordavella* sp. *Marseille* P3756, [*Clostridium*] *saccharolyticum*, and *Lachnospiraceae bacterium* GAM79 are the top bacterial species for RH1 and RH2 groups. Besides, the RH1 group hosts *Lactobacillus paracollinoides*, *Lactobacillus zymae*, *Lachnoclostridium phocaeense*, *Escherichia*

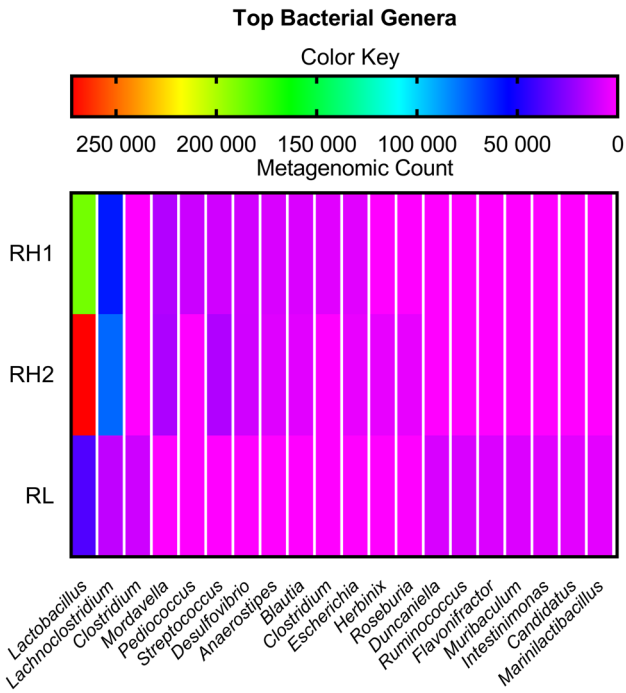

Fig. 3 The heatmap analysis of bacterial genera in the microbiota. RH1: Rat samples from the urban habitat, RH2: Rat samples from the rural habitat, RL: Laboratory rat samples

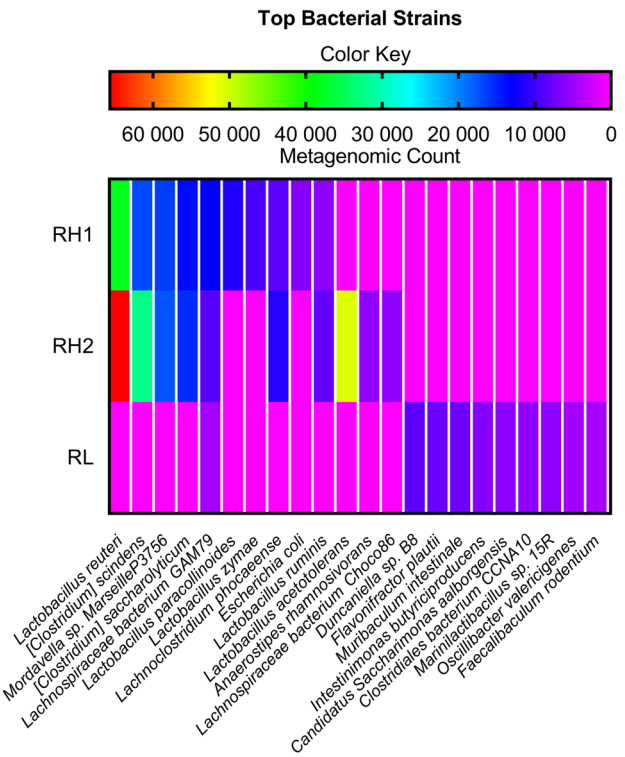

Fig. 4 The heatmap analysis of bacterial species in the microbiota. RH1: Rat samples from the urban habitat, RH2: Rat samples from the rural habitat, RL: Laboratory rat samples

coli, and *Lactobacillus ruminis* species. Distinct species of RH2 group microbiota are *Lactobacillus acetotolerans*, *Anaerostipes rhamnosivorans*, and *Lachnospiraceae bacterium* Choco86, whereas *Lactobacillus paracollinoides*, *Lactobacillus zymae*, and *Escherichia coli* species could not be detected. *L. acetotolerans* have been reported to be important in the fermentation of many traditional food beverages. It possesses the ability to produce many flavor compounds, lactic acid, acetic acid, and diacetyl as end products of carbohydrate fermentation (Liu et al. 2016). *A. rhamnosivorans* is a rod-shaped bacterium (Bui et al. 2014) capable of producing butyrate from acetic and lactic acids rather than sugars in the gut (Flint et al. 2012a). Butyrate, which can protect against cancer and ulcerative colitis (Hague et al. 1997), has also been associated with the prevention of insulin resistance and obesity in mice (Flint et al. 2012b).

The diverse species particularly attributed to RH1 and/or RH2 groups were not present in the microbiota of the RL group, except for the *Lachnospiraceae bacterium* GAM79. However, its unique bacterial profile is consisting of *Duncaniella* sp. B8, *Flavonifractor plautii*, *Muribaculum intestinale*, *Intestinimonas butyriciproducens*, *Candidatus Saccharimonas aalborgensis*, *Clostridiales bacterium* CCNA10, *Marinilactibacillus* sp. 15R, *Oscillibacter valericigenes*, and *Faecalibaculum rodentium*. The food of laboratory rats has the status of processed food of vegetable origin; therefore, their defined diet is very different compared to wild rats. For instance, *O. valericigenes* grow predominantly by producing valerate when cultivated in a glucose-rich medium. In studies on human gut microbiota, *O. valericigenes* species was found significantly higher in healthy control groups than patients diagnosed with Crohn's disease (gut inflammation) (Mondot et al. 2011). Its presence in laboratory rats' microbiota is probably due to the balanced and controlled diet of these animals.

Black rats are found in urban areas around warehouses, dwellings, and other human settlements such as roofs of buildings, suspended attics, wall voids, and underground tunnels. In rural areas, farms, barns, and rooms where crops are stored are places suitable for their nutritional needs. However, as rats can adapt to a wide variety of foods, food preference is largely determined by availability. For example, rotten food in the litter is a primary food source for rats that cannot find natural food in an urban environment (Traweger and Slotta-Bachmayr 2005). According to monitoring studies, the movement activities of rats living in urban areas are between 25 and 150 m, whereas it is in between > 260 and 2000 m in rural areas (Gardner-Santana et al. 2009). Our study areas were determined by limiting the predictable nutrient and pollutant stocks in the habitats, considering the rats' mobility. In the case of laboratory rats, they have a nutrient content consisting of many plant sources in much more sterile environments. According to these data, each rat group has a distinct microbiota pattern. It is assumed that the adaptation to different living conditions led to a different arrangement in their microbiota.

Conclusions

Environmental factors such as diet, habitat, and environmental pollutants affect the microbial composition by encouraging or suppressing the population of bacteria in the gut microbiota of rats. Many bacterial species support the intestinal barrier in which the formation of mucosa and the attachment of bacteria to intestinal cells with binding proteins creates a symbiotic habitat and provides advantages for both microorganisms and the host. Previous studies have shown remarkable effects of antibiotics, heavy metals, pesticides, and insecticides on the microbiota. These ecological pollutants which were taken with food decrease the variety of species in the microbiota and cause dysbiosis. Although the nature and dosage of the exposed contaminants have very different effects on the microbiota, they can cause significant changes in bacterial populations. By looking at the microbiota content of rats, these changes can be categorized according to certain criteria in terms of bacterial species, which will contribute to the understanding of relationships between microbiota and health. By the end, a unique microbial profile can be created for wild rats that share the same ecological environment and have similar nutritional preferences, even if the microbiota is specific to the individual organism. Identifying individual and/or community-specific microbiota profiles for rats, and monitoring them at regular intervals will be valuable as they will contain biomonitoring information on environmental pollutant levels and also the health status of the human population and/or society.

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Author contribution All authors contributed to the conception and design of the study. The final manuscript has been read and approved by all authors. Rafig Gurbanov and Tuba Yağcı designed the experiments, supervised the study, analyzed the results, and wrote the manuscript. Uygar Kabaoğlu carried out the experiments with help from Rafig Gurbanov. This study is a part of the Master of Science project of Uygar Kabaoğlu under the supervision of Tuba Yağcı and Rafig Gurbanov.

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Availability of data and material All data generated during and/or analyzed during the current study are available from the corresponding author on reasonable request. The raw sequence reads data were submitted to NCBI Sequence Read Archive (SRA), and the study was registered in NCBI BioProject and BioSample database (SubmissionID: SUB10818992; BioProject ID: PRJNA790067).

Code availability There is not any custom computer code or algorithm used to generate the results reported in the manuscript.

Declarations

Ethics approval This study was carried out with the permission (permission number: 21264211–288.04-E.3311026) of the General Directorate of Nature Conservation and National Parks affiliated to the Turkish Ministry of Agriculture and Forestry and Adnan Menderes University Experimental Animals Local Ethics Committee-ADÜ HADYEK (permission number: 64583101/2017/003). The authors declare that they have followed the ethical standards of the responsible committee.

Consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

References

Bäckhed F, Ley RE, Sonnenburg JL et al (2005) Host-bacterial mutualism in the human intestine. Science 307:1915–1920. https://doi.org/10.1126/science.1104816

Bäckhed F, Roswall J, Peng Y et al (2015) Dynamics and stabilization of the human gut microbiome during the first year of life. Cell Host Microbe 17:690–703. https://doi.org/10.1016/j.chom.2015.04.004

Binda C, Lopetuso LR, Rizzatti G et al (2018) Actinobacteria: a relevant minority for the maintenance of gut homeostasis. Dig Liver Dis 50:421–428. https://doi.org/10.1016/j.dld.2018.02.012

Breton J, Daniel C, Dewulf J et al (2013) Gut microbiota limits heavy metals burden caused by chronic oral exposure. Toxicol Lett 222:132–138. https://doi.org/10.1016/j.toxlet.2013.07.021

Bui TPN, de Vos WM, Plugge CM (2014) *Anaerostipes rhamnosivorans* sp. nov., a human intestinal, butyrate-forming bacterium. Int J Syst Evol Microbiol 64:787–793. https://doi.org/10.1099/ijs.0.055061-0

Carmody RN, Bisanz JE, Bowen BP et al (2019) Cooking shapes the structure and function of the gut microbiome. Nat Microbiol 4:2052–2063. https://doi.org/10.1038/s41564-019-0569-4

Ceylani T, Jakubowska-Doğru E, Gurbanov R et al (2018) The effects of repeated antibiotic administration to juvenile BALB/c mice on the microbiota status and animal behavior at the adult age. Heliyon 4:e00644. https://doi.org/10.1016/j.heliyon.2018.e00644

Drengenes C, Eagan TML, Haaland I et al (2021) Exploring protocol bias in airway microbiome studies: one versus two PCR steps and 16S rRNA gene region V3 V4 versus V4. BMC Genomics 22:3. https://doi.org/10.1186/s12864-020-07252-z

Fang S, Zhuo Z, Yu X et al (2018) Oral administration of liquid iron preparation containing excess iron induces intestine and liver injury, impairs intestinal barrier function and alters the gut microbiota in rats. J Trace Elem Med Biol 47:12–20. https://doi.org/10.1016/j.jtemb.2018.01.002

Flandroy L, Poutahidis T, Berg G et al (2018) The impact of human activities and lifestyles on the interlinked microbiota and health of humans and of ecosystems. Sci Total Environ 627:1018–1038. https://doi.org/10.1016/j.scitotenv.2018.01.288

Flemer B, Gaci N, Borrel G et al (2017) Fecal microbiota variation across the lifespan of the healthy laboratory rat. Gut Microbes 8:428–439. https://doi.org/10.1080/19490976.2017.1334033

Flint HJ, Scott KP, Duncan SH et al (2012a) Microbial degradation of complex carbohydrates in the gut. Gut Microbes 3:289–306. https://doi.org/10.4161/gmic.19897

Flint HJ, Scott KP, Louis P, Duncan SH (2012b) The role of the gut microbiota in nutrition and health. Nat Rev Gastroenterol Hepatol 9:577–589. https://doi.org/10.1038/nrgastro.2012.156

Gardner-Santana LC, Norris DE, Fornadel CM et al (2009) Commensal ecology, urban landscapes, and their influence on the genetic characteristics of city-dwelling Norway rats (*Rattus norvegicus*). Mol Ecol 18:2766–2778. https://doi.org/10.1111/j.1365-294X.2009.04232.x

Hague A, Singh B, Paraskeva C (1997) Butyrate acts as a survival factor for colonic epithelial cells: Further fuel for the in vivo versus in vitro debate. Gastroenterology 112:1036–1040. https://doi.org/10.1053/gast.1997.v112.agast971036

Helmink BA, Khan MAW, Hermann A et al (2019) The microbiome, cancer, and cancer therapy. Nat Med 25:377–388. https://doi.org/10.1038/s41591-019-0377-7

Indiani CMDSP, Rizzardi KF, Castelo PM et al (2018) Childhood obesity and firmicutes/bacteroidetes ratio in the gut microbiota: a systematic review. Child Obes 14:501–509. https://doi.org/10.1089/chi.2018.0040

Jankovská I, Miholová D, Langrová I et al (2009) Influence of parasitism on the use of small terrestrial rodents in environmental pollution monitoring. Environ Pollut 157:2584–2586. https://doi.org/10.1016/j.envpol.2009.04.008

Licht TR, Bahl MI (2019) Impact of the gut microbiota on chemical risk assessment. Curr Opin Toxicol 15:109–113. https://doi.org/10.1016/j.cotox.2018.09.004

Liu J, Deng Y, Peters BM et al (2016) Transcriptomic analysis on the formation of the viable putative non-culturable state of beer-spoilage *Lactobacillus acetotolerans*. Sci Rep 6:36753. https://doi.org/10.1038/srep36753

Lleal M, Sarrabayrouse G, Willamil J et al (2019) A single faecal microbiota transplantation modulates the microbiome and improves clinical manifestations in a rat model of colitis. EBioMedicine 48:630–641. https://doi.org/10.1016/j.ebiom.2019.10.002

Magne F, Gotteland M, Gauthier L et al (2020) The Firmicutes/Bacteroidetes ratio: a relevant marker of gut dysbiosis in obese patients? Nutrients 12(5):1474. https://doi.org/10.3390/nu12051474

Manichanh C, Reeder J, Gibert P et al (2010) Reshaping the gut microbiome with bacterial transplantation and antibiotic intake. Genome Res 20(10):1411–1419. https://doi.org/10.1101/gr.107987.110

Matthews JA (2014) Diversity indices. In Encyclopedia of environmental change (vol 1, pp 290–291). SAGE Publications, Ltd. https://doi.org/10.4135/9781446247501.n1100

Mondot S, Kang S, Furet JP et al (2011) Highlighting new phylogenetic specificities of Crohn's disease microbiota. Inflamm Bowel Dis 17:185–192. https://doi.org/10.1002/ibd.21436

Razavi AC, Potts KS, Kelly TN, Bazzano LA (2019) Sex, gut microbiome, and cardiovascular disease risk. Biol Sex Differ 10:29. https://doi.org/10.1186/s13293-019-0240-z

Rizzatti G, Lopetuso LR, Gibiino G et al (2017) Proteobacteria: a common factor in human diseases. Biomed Res Int 2017:9351507. https://doi.org/10.1155/2017/9351507

Rothschild D, Weissbrod O, Barkan E et al (2018) Environment dominates over host genetics in shaping human gut microbiota. Nature 555:210–215. https://doi.org/10.1038/nature25973

Shahi SK, Zarei K, Guseva N, v, Mangalam AK, (2019) Microbiota analysis using two-step PCR and next-generation 16S rRNA gene sequencing. J Vis Exp. https://doi.org/10.3791/59980

Sune D, Rydberg H, Augustinsson ÅN et al (2020) Optimization of 16S rRNA gene analysis for use in the diagnostic clinical microbiology service. J Microbiol Methods. https://doi.org/10.1016/j.mimet.2020.105854

Traweger D, Slotta-Bachmayr L (2005) Introducing GIS-modelling into the management of a brown rat (*Rattus norvegicus* Berk.) (Mamm. Rodentia Muridae) population in an urban habitat. J Pest Sci 78:17–24. https://doi.org/10.1007/s10340-004-0062-5

Wood DE, Salzberg SL (2014) Kraken: Ultrafast metagenomic sequence classification using exact alignments. Genome Biol 15:R46. https://doi.org/10.1186/gb-2014-15-3-r46

Wopereis H, Oozeer R, Knipping K et al (2014) The first thousand days — intestinal microbiology of early life: establishing a symbiosis. Pediatr Allergy Immunol 25:428–438. https://doi.org/10.1111/pai.12232

Zhang F, Zheng W, Guo R, Yao W (2017) Effect of dietary copper level on the gut microbiota and its correlation with serum inflammatory cytokines in Sprague-Dawley rats. J Microbiol 55:694–702. https://doi.org/10.1007/s12275-017-6627-9