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“An impact of Wi-Fi irradiation on the gut microbiome of rats”

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Article

Keywords: Wi-Fi, radiation, electromagnetic field, microbiome, gut, bio-effects

Posted Date: March 17th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2673000/v1

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Abstract

The research on the bio-effects caused by Wi-Fi radiation has been mainly focused on the reproductive, nervous, and cardio-vascular systems. However, a comprehensive investigation of the influence of Wi-Fi on the gut microbiome has not been done yet. The ultimate goal of the study was to investigate the effect of Wi-Fi radiation on the gut microbiome of rats. The Wistar rats have been subjected to the Wi-Fi radiation. Changes in the microbiome composition were studied over 30 days after irradiation. The DNAs were isolated from the faeces samples and sequenced. A complete bioinformatics analysis was carried out. It was found out that on the 14th day of Wi-Fi irradiation, the biodiversity of the intestinal microflora decreased. We observed a significant decrease in the number of Bifidobacteria from the first day of the experiment. Wi-Fi exposure caused the growth of bacteria of the genus Helicobacter. In addition, there was also an increase in the number of opportunistic pathogens of the Flavobacteriia class. The findings indicate the ability of Wi-Fi radiation to modulate the activity of gut micro-organisms that might affect the health status in the long perspective.

Introduction

The impact of non-ionising electromagnetic fields (EMFs) on human health has been a topic of numerous studies over the last three decades. In fact, the knowledge about EMF-caused biological effects has been constantly accumulated and analysed worldwide [1–3].

The research has covered a wide range of electromagnetic frequencies: from zero up to 5 GHz. The main concern of the public has been associated with wireless networking technology, such as mobile telephony and Wi-Fi. Wi-Fi,(WLAN Wireless Local Area Network), is the technology for the wireless connection of various devices [4]. Such a technology has been widely used mainly for providing Internet access in offices, public places, universities, transport, etc. Nowadays, there are different types of Wi-Fi standards. The IEEE 802.11 Wireless LAN (WLAN) & Mesh is the leading standard for most countries. The type of frequencies of Wi-Fi depend on the countries' standards and approved protocols, including 900 MHz, 2.4 GHz, 3.65 GHz, 5.0 GHz, 5.9 GHz, 6 GHz, and 60 GHz frequencies.

Up to date, there is a range of reports on the bio-effects caused by Wi-Fi radiation, including oxidative stress [5–7], sperm damage [8–11], neural development [6,12], apoptosis [5,7], hormonal misbalance [7,13], etc. The research was predominantly focused on the reproductive, nervous and cardio-vascular systems only [14]. However, the comprehensive investigation of the influence of Wi-Fi radiation on the gut microbiome has not been done yet.

The gut microbiome (microbiota) is implicated in the regulation of human health and metabolism. The microbiome has been demonstrated to have an impact on all systemic processes, including, but not limited to, immune and brain functions [15,16]. The gut microbiome and the immune system are intricately linked, with each influencing the other in complex ways. A healthy gut microbiome helps to maintain a strong immune system, while disruptions to the gut microbiome can lead to immune system dysfunction

[17–19]. The gut microbiome helps to educate the immune system and promote tolerance to harmless antigens. The microbes in the gut produce various molecules that stimulate the immune system, helping to develop and maintain a balanced immune response. The gut microbiome also helps to prevent the overactive immune responses that can lead to chronic inflammation and autoimmune diseases.

The microbiome balance can be disrupted by a number of factors, including diet, antibiotics, and other environmental factors. It was shown that the ionizing radiation can disturb the structure and function of the gut microbiome, leading to a range of health problems [20,21]. Ionizing radiation can damage the DNA of gut microbes, leading to pathological changes in their composition and activity. This can result in the loss of beneficial bacteria and the overgrowth of harmful pathogens, finally, it can lead to imbalance in the intestinal microbiome. Such an imbalance might have a variety of effects on human health, including impaired digestion, weakened immune response, and increased risk of infections and other health problems [19,22,23].

Ionizing radiation can have systemic effects on the human body, affecting the entire gut-liver-blood system. This can further impact the gut environment, reducing the availability of essential nutrients and fibre, and altering the production of hormones, essential vitamins, and other signalling molecules. Despite the massive data about the effect of ionizing radiation, there is a lack of information on the impact of non-ionizing and non-thermal electromagnetic radiation on the microbiota, including EMFs in the Wi-Fi radio-frequency range. This study aimed to shed the light on the reaction of the gut microbiome to long-term Wi-Fi exposure. We utilized the animal model (rats) to analyse the changes in microbiome structure caused by Wi-Fi irradiation.

Results

The microbial signature of the faecal microbiome of rats after exposure to Wi-Fi showed a trend of decreasing diversity and decreased to the lowest value by day 14 (Fig. 1a). Thus, a significant decrease in diversity ($p = 0.011$) was observed on day 14 after exposure.

Intergroup comparison of biodiversity revealed differences at the phylum level. For example, the relative abundance of representatives of Actinobacteria phylum decreased on average in the first 3 days. The abundance of Bacteroidetes phylum and Unclassified bacteria also decreased (Fig. 1b). At the genus level, from days 1 to 3, the abundance of Prevotella and Selenomonas increased in the faecal microbiota of rats after exposure to Wi-Fi, while the content of Faecalibacterium tended to decrease. On the third day, the relative abundance of genera belonging to Bacteroidales, Clostridiales and Ruminococcaceae, S24-7 (Muribaculaceae) decreased too (Fig. 1d). Principle Coordinates Analysis (Fig. 1c) of Bray-Curtis found a not significant (ANOSIM: r = 0.03, $p = 0.154$) divergence in microbial communities between the control and Wi-Fi exposed groups.

In order to determine the effect of Wi-Fi exposure on the composition and structure of the rat gut microbiome, the Wilcoxon signed rank test (both at the level of genera and at the OTU level) was

conducted to compare the abundance of different taxonomic groups.

To identify the most represented taxa in the intestinal microbiota before and after exposure, the linear discriminant analysis (LDA) effect size (LEfSe) method was used (Fig. 2). The results showed that bifidobacteria significantly predominated in the control group. The Wi-Fi exposure caused an increase in the amount of coccal flora, including micro-organisms types related to the genus Coprococcus, Anaerosinus, Candidatus Arthromitus, and Ruminococcus. The number of cyanobacteria belonging to the class Streptophyta has also increased. In addition, we observed the growth of the number of opportunistic pathogens of the Flavobacteriia class (Weeksellaceae, genus Elizabethkingia) after 14 days of exposure. The LDA analysis demonstrated a rise in the number of Lactobacillales bacteria on the 30th day after the Wi-Fi irradiation. Such a type of bacteria was predominately presented by Lactobacillus ruminis and Streptococcus genus.

Abundance pattern analysis revealed that the levels of three bacterial species (Helicobacter sp., Bifidobacterium animals, Bifidobacterium pseudolongum) taken from faecal samples after exposure from days 1 to 30 were significantly different (Fig. 3). In addition, Fig. 3 shows that the relative abundances of the 3 taxa in the faecal bacteriome are different at the points of analysis. For example, the abundance of Helicobacter sp. increases after exposure to Wi-Fi from days 1 to 14, then a decrease was observed by day 21. In contrast, the relative abundance of taxa of the genus Bifidobacterium (compared to the control group) decreased during the first 14 days. However, a partial restoration of their concentration was detected at 21st day.

Heat tree analysis was used to display the group relative abundance of microbial communities. Supplement Fig. 1 (heat trees) illustrating taxonomic differences between study groups and control. The figures show that the relative abundance of Bifidobacteria and Fusobacteria (compared to the control) was significantly reduced ($p = 0.05$), while the number of Oligosphaerales was increased on the 21st day.

Taking into account the differences in the microflora structure, we made a prediction of the potential functionality of gene content based on a comparison between the relative abundance and reference genomes of the taxa present using the PICRUSt2 tool. Based on the analysis of MetaCyc database, a total of 7 metabolic and biosynthetic pathways were found to differ when pairwise compared between groups before and after Wi-Fi exposure (Fig. 4). It was shown that most of the altered functional pathways belonged to the main categories: metabolism of cofactors and vitamins, amino acid metabolism, energy metabolism, biosynthesis of other secondary metabolites, lipid metabolism, drug resistance.

Discussion

Recent research has shown that the gut microbiome can have a significant impact on various aspects of human health, including digestion, immune system function, and mental health [24–26]. Digestion is one of the most important functions of the gut microbiome. The microbes in the gut break down undigested

food particles, producing short-chain fatty acids and other metabolites that are essential for human nutrition [27–29]. They also help to regulate the pH of the gut, promoting healthy digestion and preventing harmful bacteria from overgrowing.

The gut microbiome plays a key role in the development and function of the human immune system [17,30]. Microbes in the gut help to educate the immune system and promote tolerance to harmless antigens, while also helping to prevent the overactive immune responses that can lead to chronic inflammation and autoimmune diseases. The gut microbiome also helps to regulate the gut-liver axis, which is important for the metabolism of toxins and other harmful substances in the body.

In addition to its effects on physical health, the gut microbiome has also been linked to mental health and behaviour [24,31]. Research has shown that the gut microbiome can affect neurotransmitter production, inflammation, and other processes in the brain that play a role in mood and behaviour [32–34]. For example, imbalances in the gut microbiome have been linked to anxiety, depression, and other mood disorders. The results of a range of in vivo studies demonstrated that faecal transplant can have an impact on neuropsychiatric diseases. The links between the gut microbiome and the brain ('the gut-brain axis') have been described for a number of conditions, including depression and autism.

Up to date, there is a limited research has been done on the effects of Wi-Fi radiation on the intestinal microflora. It was demonstrated that exposure to electromagnetic fields led to a decrease in the abundance of Butyricicoccus, Lachnospiraceae, Anaerotruncus, Bilophila, Tuzzerella in the intestines of mice [35]. Taheri et al. showed that radio-frequency radiation emitted by a Wi-Fi router affects the growth rate and antibacterial susceptibility of intestinal bacteria [36].

Our findings indicate that Wi-Fi radiation led to a change in the biodiversity and taxonomic composition of the intestines of rats. The post-exposure microbiome was enriched in proteolytic microflora and depleted of several beneficial representatives of beneficial bacteria. Such a disturbance of the microflora is typical for a range of pathological conditions, such as inflammatory bowel disease (IBD). There is a growing body of evidence that the changes in the composition and diversity of the gut microbiome are key factors leading to the development of IBD [37–39]. For instance, Fourie N.H. et al. observed similar changes in gut microbiota composition in the stress-induced IBD model [40].

We observed gut microflora disturbances on the first day following the Wi-Fi exposure. Such changes were consistent throughout the experimental period. However, the maximum differences in the structural diversity of the microbial composition of the intestinal lumen were observed on day 14 after exposure.

Further observation revealed a trend towards restoration of the biodiversity of prokaryotic organisms (Fig. 1a). The details of all changes are shown in Fig. 2 (b,c,d). Figure 1 illustrates the biggest changes affected Actinobacteria phylum, where the decrease in abundance was observed in the first two weeks. The main contribution to such a decrease was made by the genus Bifidobacterium.

On the 30th day after exposure, an increase in the biodiversity of microorganisms of the faecal microbiome of rats was observed. Here, an increase in the abundance of Lactobacillales taxa was detected. It may indicate a trend towards the restoration of the bacterial richness of the intestinal microflora. Structural taxonomic changes in the intestinal bacterial community of the studied group of rats were associated with changes in the microbiome response to Wi-Fi radiation. Metabolic functions associated with energy, amino acid metabolism, biosynthesis of other secondary metabolites, according to the prediction of functional abundance, were reduced in the microbiome of animals exposed to Wi-Fi. Moreover, Wi-Fi irradiation increased the ability of microbiota to metabolize thiamine and fatty acid biosynthesis. Such a dysregulation in fatty acid metabolism has been commonly associated with Irritable bowel syndrome (IBS) and other inflammatory bowel disorders [40,41].

Conclusions

The study's results showed that the Wi-Fi radiation caused a decline in the gut biodiversity of rats. It turned out that radiation has a different effect on various types of intestinal microorganisms. A significant decrease in Bifidobacteria (Bifidobacterium animals and Bifidobacterium pseudolongum) was detected on the first day of the experiment. The growth of Helicobacter colonies was observed during the first week, then the values returned to normal ones. On day 14, there was also an increase in the number of opportunistic pathogens belonging to the Flavobacteriia class (Weeksellaceae, genus Elizabethkingia). It must be noted that the observed dysregulation in fatty acid metabolism and changes in microbiome structure is typical for the symptoms of inflammatory bowel disease, irritable bowel syndrome, and other bowel pathologies.

The findings indicate the ability of Wi-Fi radiation to modulate the activity of gut micro-organisms that might affect the health status in the long perspective. However, further intensive studies are required to validate the clinical relevance and potentially harmful effects for humans.

Materials And Methods

Animal studies

14 male Wistar rats (weight: 300–450 g), were used for the experiments. Under controlled standard laboratory conditions (22°C, normal humidity, and a 12/12-hour light/dark cycle), the rats were housed in cages (one animal per cage) with free access to water and standard rodent food. Physical examination and laboratory testing were used to first determine whether the animals were unwell.

The experiments were carried out at the Research Institute of Fundamental and Applied Medicine named after B. Atchabarov.

Ethical issues

All procedures for in vivo study were conducted according to the Helsinki declaration of protection of vertebrate animals (1975; revised version of 2008). The protocol of the in vivo study was approved by the Local Ethical Committee of the Kazakh National Medical University (protocol No. 3 (88) of April 30, 2021). The study is reported in accordance with ARRIVE guidelines [42].

All methods were performed in accordance with the relevant guidelines and regulations. No human participants were involved in the study.

Exposure set-up

For the exposure the animals, the commercial Wi-Fi device was utilized (TP-LINK, China, model TD-W9970 V3, certification CE / RoHS). The technical characteristics: IEEE Standards IEEE 802.3 /802.3u, frequency 2.400-2.4835 GHz, transmission power < 20 dBm (< 100 mW) (EIRP), indoor antenna gain 2 x 4dBi. The 7 rats were exposed to Wi-Fi radiation 24 h per day (during 30 days). Control group consisted of 7 rats unexposed to Wi-Fi radiation.

To monitor undesirable temperature effects caused by the Wi-Fi radiation, we employed specially calibrated fiber optic sensor TS3 thermocouple (OPTOCON, Germany) [43,44]. "FOTEMP-Assistant" software (OPTOCON, Germany) running on the Microsoft Windows operating system was utilized for temperature monitoring and analysis. Such a type of thermocouple provides a stable temperature measurement in an EM / Radiofrequency environment.

Sample preparation, processing, and sequencing

Animal faecal samples were collected in sterile centrifuge tubes every day. Genomic DNA from faecal samples was extracted immediately, using the ZymoBIOMICS DNA Miniprep Kit (Zymo Research, D4300). A qualitative control of DNA isolation was performed by OD260/280 nanodrop and electrophoresis in a 1% agarose gel. The concentration and purity of each DNA sample were determined using an Invitrogen Qubit 3.0 Fluorometer (Invitrogen, Carlsbad, California, United States). Sterile water served as a negative control. Sequencing was performed on the Illumina NovaSeq 6000 platform at the laboratory of Novogene (Beijing, China) following the standard Illumina protocols.

Data processing and statistics

Analysis of the microbiota composition was performed using the microbiome analysis pipeline QIIME 2 v2022.11 [45]. Alpha-diversity metrics (observed features and Faith's Phylogenetic Diversity) [46], beta diversity metrics weighted UniFrac [47], unweighted UniFrac [48], Jaccard distance, and Bray-Curtis dissimilarity), and Principle Coordinate Analysis (PCoA) were estimated using q2-diversity after samples were rarefied (subsampled without replacement) to 900 sequences per sample. Differences in taxa at different levels were analysed by the linear discriminant analysis (LDA) of effect size (LEfSe) [49]. The abundance of taxa was considered significantly different if their differences had a p value < 0.05 and an LDA score (log10) > 2. The heat tree analysis was performed using the online MicrobiomeAnalyst

software [50]. All statistical analyses were performed in Rv. 4.2.1 using 'Tidyverse r package' [51]. For richness estimation, 'Phyloseq r package'. The plots were made in R using the 'ggplot2 package' [52].

Functional capabilities of the oral microbiome

Functional metagenomes were predicted based on the 16S rRNA sequencing data of the oral microbiome using PICRUSt2 (phylogenetic investigation of communities by reconstruction of unobserved states) v2.5.0 with default parameters [53]. Briefly, the AVSs were placed into a reference tree (NSTI cut-off value of 2) containing 20,000 full 16S rRNA sequences from prokaryotic genomes, which was then used to predict individual gene family copy numbers for each AVS. The predictions are based on Kyoto Encyclopaedia of Genes and Genomes (KEGG) orthologs (KO). The produced KEGG orthologs (KOs) were mapped to the KEGG module annotation downloaded on 04/01/2022 from the KEGG BRITE database [54].

Experimental design

Stool samples for metagenome analysis were collected before Wi-Fi exposure (the control group), and 1, 3, 7, 14, 21, and 30 days after exposure. For the analysis of the microbial community the 16S rRNA gene was selected. The depth of coverage was at least 87 534 readings per sample. All sequence sequences were compared with the Greengenes database [55,56] using the qiime2 bioinformatics process. A total of 14798 OTUs were used for the final analysis. Average counts reads per sample 126701. For alpha diversity analysis, qiime2r was used which showed a decrease in the Shannon Bacterial Diversity Index score after Wi-Fi exposure.

Declarations

Authors contribution

Timur Saliev: Supervision, Methodology, Formal analysis, Investigation, Writing – original draft;

Samat Kozhakhmetov: Conceptualization, Resources, Analysis, Writing – review & editing;

Madiyar Nurgaziyev: Investigation, Resources, Writing – review & editing;

Zharkyn Jarmukhanov: Statistics, Methodology, Formal analysis, Investigation;

Shamil Mureyev: Investigation, Resources, Writing – review & editing;

Almagul Kushugulova: Conceptualization, Resources, Analysis, Writing – review & editing;

Timur Fazylov: Animal handling, Research, Analysis;

Ildar Fakhradiyev: Investigation, Resources, Writing – review & editing

The authors are grateful to the S.D. Asfendiyarov Kazakh National Medical University for the administrative and technical support. The authors would like to thank the Ministry of Agriculture of the

Republic of Kazakhstan for funding this research work (grant “Development of technologies using new strains of beneficial microorganisms, enzymes, nutrients, and other kits in the production of special dietary food products” IRN: BR10764998).

Funding

The study did not receive any external or internal funding.

Declaration of competing interest

The author(s) declare no competing interests.

Data availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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Figures

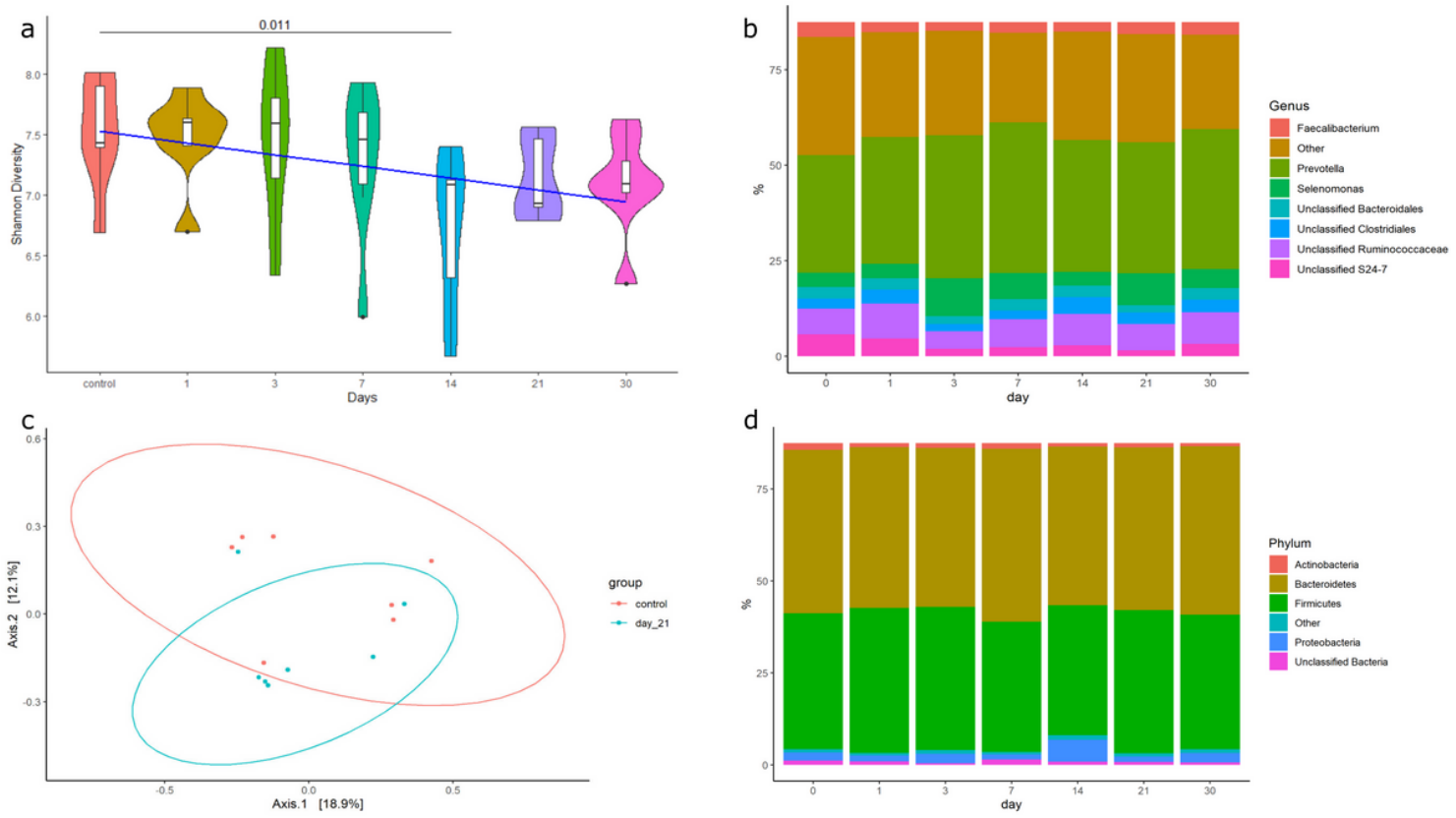


Figure 1

Evaluation of the diversity of the rat's faecal microbiota before and after exposure to Wi-Fi. a) Alfa diversity analysed by the Shannon index; b) Boxplots display the median value, the first (25 %) and third (75 %) quartiles with whiskers from 1.5 IQR (interquartile range) minimum to maximum on phylum level. x-axis is the value of the Shannon biodiversity index; y-axis - days of analysis of faecal samples; c) PCoA

of abundance based Bray-Curtis distances of the colonic microbiome from control (red) and Wi-Fi (blue) rats after 14 days; d) Boxplots display the median value, the first (25 %) and third (75 %) quartiles with whiskers from 1.5 IQR (interquartile range) minimum to maximum on phylum level. x-axis is the value of the Shannon biodiversity index; y-axis - days of analysis of faecal samples.

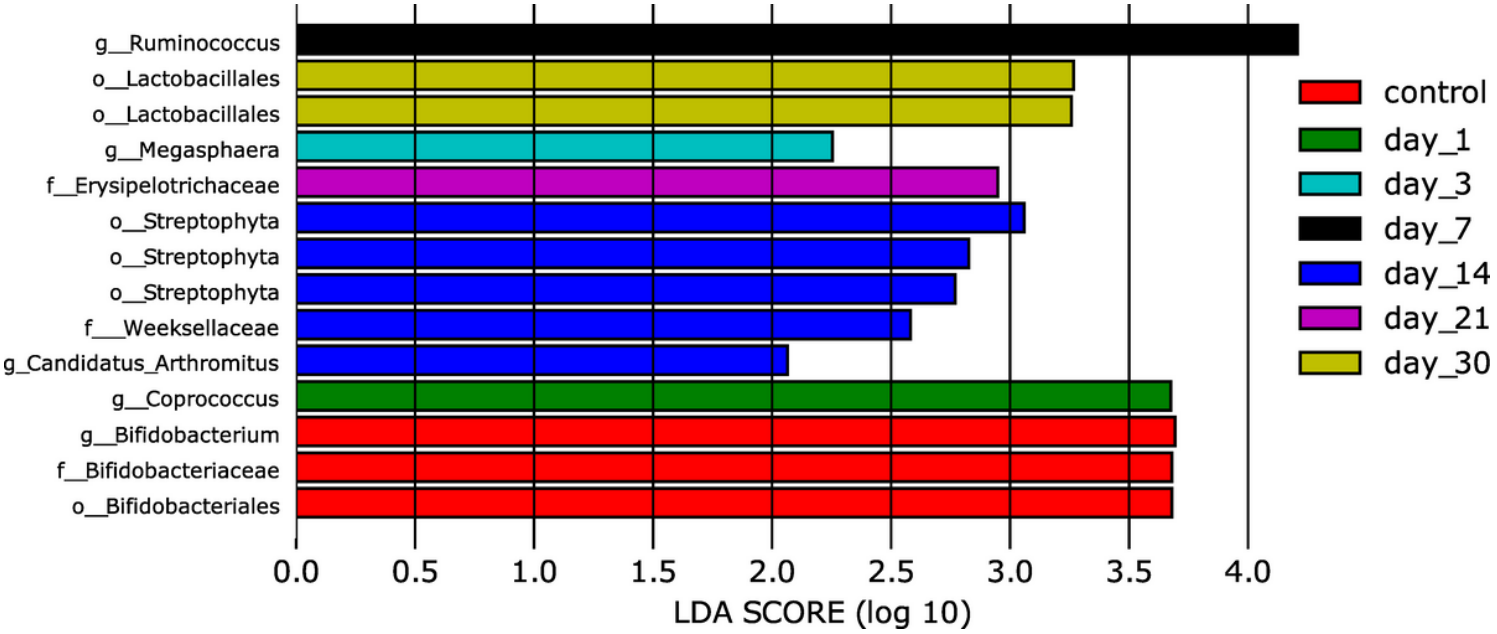


Figure 2

Linear discriminant analysis (LDA) effect size (LEfSe). The list of characteristics with statistical and biological importance that change between conditions of interest is initially provided by LEfSe, which ranks the characteristics according to the size of the effect. To find features having significant differential abundance with respect to the class of interest, the non-parametric factorial Kruskal-Wallis (KW) sum-rank test is utilized. The (unpaired) Wilcoxon rank-sum test is then used to conduct a series of pairwise tests among subclasses to examine biological consistency. The final phase of LEfSe is to estimate the effect size of each differentially abundant feature using LDA.

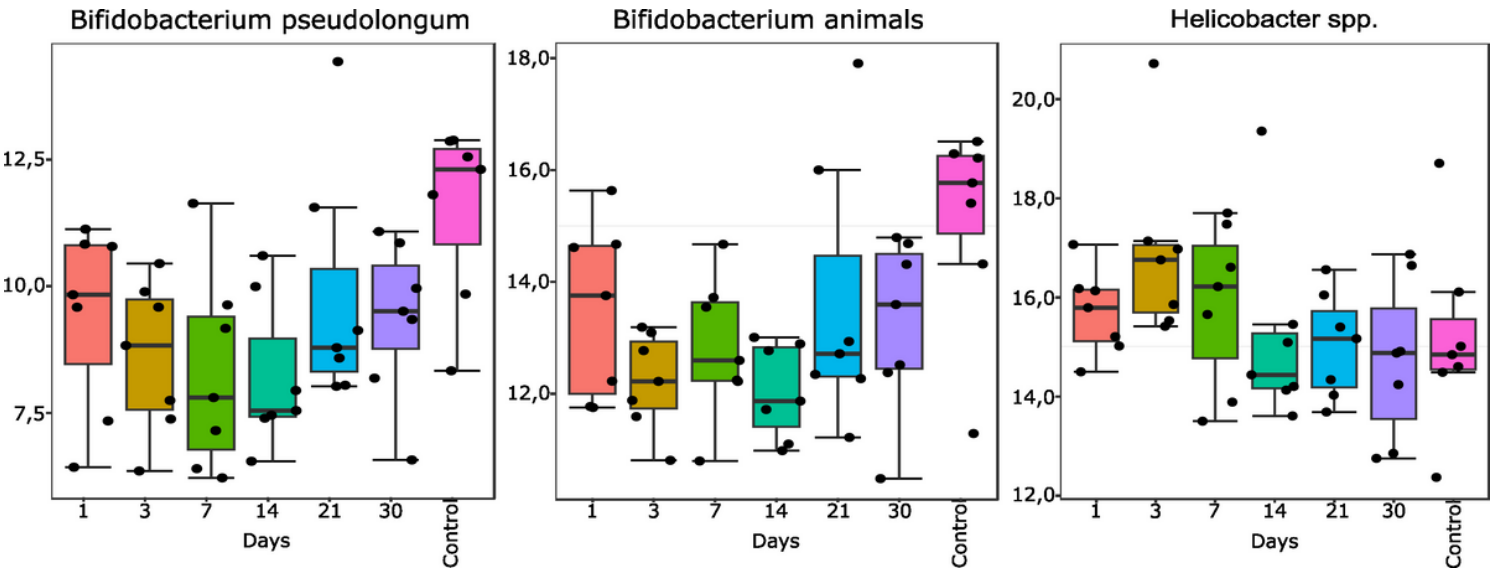


Figure 3

Box plots showing the relative abundance of differing bacteria in rat faecal samples before and after Wi-Fi exposure.

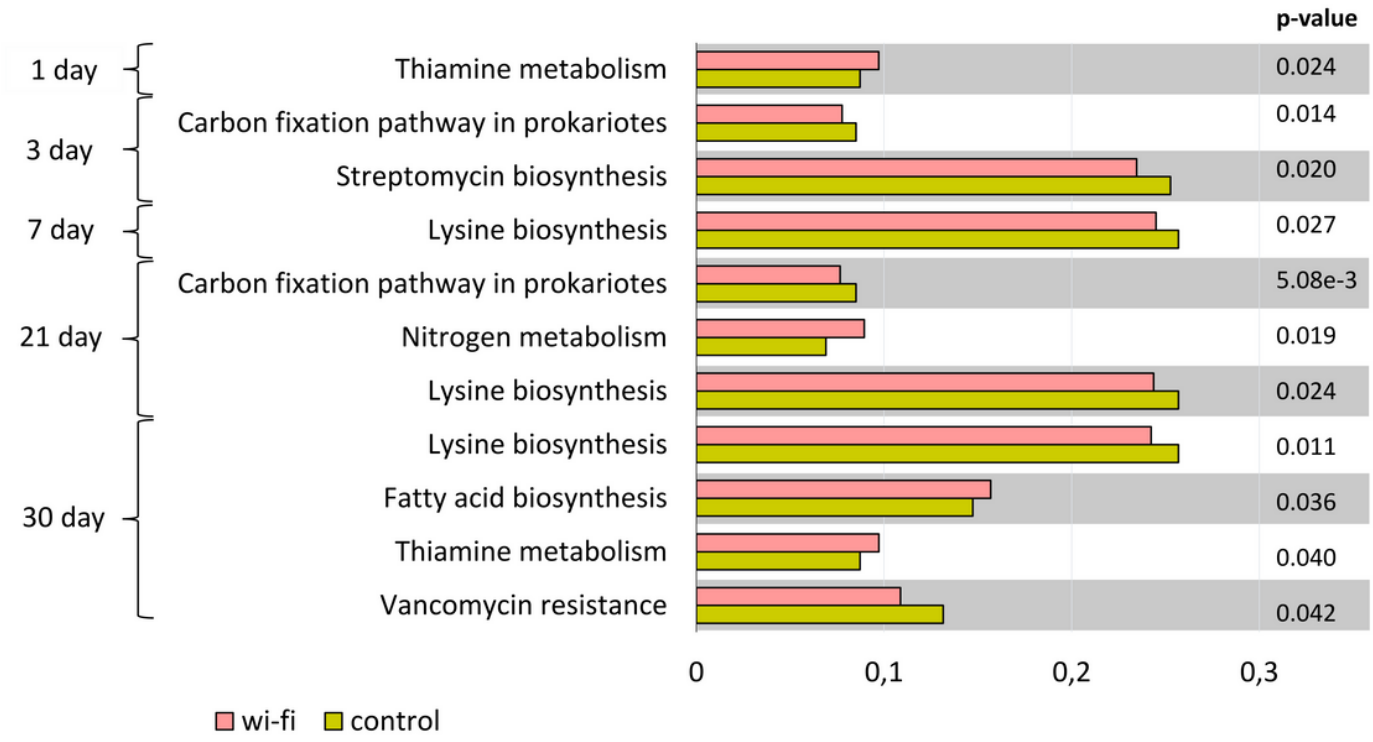


Figure 4

Predicted functional metagenomics pathways of the rat's faecal microbiome. Red indicates the Wi-Fi group, and yellow indicates the control group.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryFigure1.docx