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ORIGINAL PAPER

The effect of young blood plasma administration on gut microbiota in middle-aged rats

Taha Ceylani[1] · **Hikmet Taner Teker**[2]

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Abstract

Numerous in-depth studies continue to reveal the many benefits of gut microbiota and young blood plasma administration. Dysbiosis, which occurs in the intestinal microbiota, especially in the aging process, is associated with many metabolic and cognitive disorders. Therefore, many studies aim to reverse the dysbiosis that occurs. There are also studies showing that young blood plasma application reverses the effects of aging at the level of many tissues and organs. Today, while research continues to reveal all the benefits of young blood plasma application in terms of health, blood plasma centers are also being established. In this study, we aimed to reveal the impact of young blood plasma, administered for 1 month, on the intestinal microbiota of middle-aged rats. After detailed metagenome analysis, alpha diversity indices demonstrated greater bacterial richness in the microbiota of plasma-administered rats compared with control rats. In addition, the Firmicutes/Bacteroidetes ratio was significantly diminished in plasma group microbiota, confirming possible rejuvenation properties of young plasma. Furthermore, increased counts of *Bifidobacterium longum, Coprococcus catus,* and *Romboutsia ilealis* species were measured in plasma-administered rats. The study revealed many fluctuations in different bacterial taxonomic units of the microbiota that could be valuable in future research on blood-based anti-aging treatments.

Keywords Young blood plasma · Gut microbiota · Metagenome · Alpha diversity · Middle-aged rat

Introduction

The gut microbiota, which is considered an extra organelle, provides many advantages to its host. These intense bacterial groups, which are metabolically very active, play an important role in the continuity of vital conditions such as the development and effectiveness of the innate and adaptive processes of the immune system, the regulation of bowel movements, the provision of digestive balance, the absorption of nutrients, and the distribution of fat molecules (O'Hara and Shanahan 2006; Chaplin et al. 2018; Seo and Holtzman 2020). For example, gut bacteria aid in the digestion of most dietary fibers that the host cannot use directly, such as cellulose and starch. Acetate, butyrate, and propionate are examples of short-chain fatty acids (SCFAs), which are the main end products of fermentation. In addition to providing energy to colonic epithelial cells, these products play important roles in the host's energy homeostasis, inflammation, and brain activity (Chi et al. 2017). As for the species that make up the gut microbiota, we know that the adult microbiota comprises more than 1000 species and approximately 7000 phyla. Despite the diversity of species within the human microbiota, Bacteroidetes and Firmicutes are mainly found in bacterial strains that make up more than 90% of the gut microbiota, while Proteobacteria, Actinobacteria, Fusobacteria, and Verrucomicrobia strains occur in relatively small amounts and can provide important metabolites and functions for health, although in low proportions (Cryan and Dinan 2012; Kim and Jazwinski 2018).

The intestinal microbiota is affected by many factors, such as complications, injuries, attack by pathogens, pollution, hormonal variations, genetic predisposition, antibiotics, unbalanced diet, diseases, and old age (Pierantozzi et al.

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Taha Ceylani and Hikmet Taner Teker contributed equally to this work.

✉ Taha Ceylani
t.ceylani@alparslan.edu.tr

[1] Department of Food Quality Control and Analysis, Muş Alparslan University, Muş, Turkey

[2] Department of Medical Biology and Genetics, Ankara Medipol University, Ankara, Turkey

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2001; Hildebrandt et al. 2009; Hashim et al. 2014; Clarke et al. 2014; Shoemark and Allen 2015; Luczynski et al. 2016; Ceylani et al. 2018). Dysbiosis occurs when intestinal microbiome homeostasis is disrupted by the effects of the aging process. Many clinical reports have shown that gut microbiota dysbiosis can directly cause inflammatory bowel disease and infectious colitis (Nishida et al. 2018). Dysbiosis is also considered to be one of the most important factors associated with other diseases such as obesity, necrotizing enterocolitis, type I and type II diabetes, and colon cancer (Kim and Jazwinski 2018; Mangiola et al. 2018). Dysbiosis also causes the functional abilities of intestinal epithelial cells to decrease or even disappear (Hemarajata and Versalovic 2013). Paneth cells, goblet cells, enteroendocrine cells, and absorbent epithelial cells in the intestinal tract play a role in maintaining the microbial balance with the help of mucus and antimicrobial peptides they produce while keeping intestinal permeability under control and preventing chronic inflammation, which is considered one of the causes of aging (Salzman et al. 2007; Gallo and Hooper 2012). Although rebiosis, which is the re-establishment of the natural microbiota, seems to be the most effective remedy against these conditions, there are very limited options, such as the use of probiotics to support this regeneration (Lloyd-Price et al. 2016; Karadağ et al. 2022).

Today, young blood plasma application has been one of the approaches used to eliminate the diseases caused by aging and even reverse aging. Studies so far have shown that young blood plasma administration has positive effects on survival in older animals (Kennedy et al. 2014). Exposure to young blood through heterochronic parabiosis has been shown to improve stem cell function in muscle, liver (Conboy et al. 2005; Brack et al. 2007), spinal cord (Ruckh et al. 2012), and brain (Villeda et al. 2011). Aged mice transfused with blood from young individuals demonstrate age-related reductions in cardiac hypertrophy and ventricular myocyte size (Loffredo et al. 2013). In another study, older people who shared younger blood showed age-related declines in pancreatic-cell proliferation, which returned to youthful levels just 2–3 weeks after sharing young blood, as well as improvements in kidney aging parameters (Huang et al. 2018). In a tibia fracture repair model, the regeneration capacity of young blood was also revealed (Castellano 2019). In this area, the effects of young blood, by unknown mechanisms, have been channeled beyond the blood–brain barrier to improve age-related brain dysfunction, with observations of increased neurogenesis in adults and synaptic plasticity. Improved hippocampal and therapeutic effects have been shown at the cognitive level in the brain (Villeda et al. 2014). Impaired autophagy-dependent liver damage has also been linked to aging. However, the administration of young plasma moderately reduced liver damage by restoring the non-functioning autophagy process in old rats. Therefore, juvenile plasma has been suggested to have a beneficial role in the treatment of age-related organ degradation (Liu et al. 2018). It has been reported that non-proliferative and antibody-secreting plasma cells have been present in the human gut for decades and provide long-term immunity to gastrointestinal pathogens (Landsverk et al. 2017).

As stated above, molecules circulating in young blood can be used to limit or reverse the direction of aging in various organs, since they have a healing effect on aging cells and tissues (Shetty et al. 2018). However, there is no scientific report presented yet on how young blood plasma application affects the shaping of intestinal microbiota. Decreasing diversity of the gut microbiota, generally associated with adverse outcomes in adults, has been linked to aging (Biagi et al. 2010). In terms of specific taxa, some studies have observed a decrease in beneficial Lactobacillus and Bifidobacterium with aging (Hébuterne 2003). The changes in the Firmicutes to Bacteroidetes ratio (F/B ratio) have been reported in three age groups: infants, adults, and the elderly (Mariat et al. 2009). Therefore, it was the subject of our curiosity to see how the young blood plasma application would affect the intestinal microbiota. We decided to use metagenome analysis to look at how the microbiota changed after young blood plasma was given to middle-aged rats for a month.

Materials and methods

Animal models

Male Sprague Dawley rat species was used as a model organism in the study. Middle-aged rats (12 months, $n=7$) were treated with pooled plasma (0.5 ml per day for 30 days, intravenously into the tail vein) collected from young (5 weeks, $n=45$) rats. Individuals in the control group ($n=7$) did not receive any treatment. One day after the end of the application, the animals in the experimental and control groups were slightly stunned by treatment with ether and sacrificed. The intestinal tissue cecum regions were taken with their contents shocked on dry ice and left in the − 80 °C deep freezer until the time to be studied. For the metagenome analysis, all of the cecum samples from both the experimental and control groups were used. All animals were housed under standard animal care conditions and had free access to food and water. Our study was carried out with the approval of the Ethics Committee from the Kobay DHL A.S. Local Ethics Committee on Animal Experiments (Approval number: 506-11/09/2020).

Plasma collection and treatments

Animals were rendered unconscious by short-term treatment with ether before blood samples were taken. Pooled rat plasma was collected by intracardial bleed at the time of euthanasia. Plasma was prepared from blood collected with EDTA, followed by centrifugation at 1000*g*. The plasma was denaturated by heating it for 2–3 min at 95 °C, followed by a short spin at 1000*g*. All plasma aliquots were stored at − 80 °C until use. Before it was given, EDTA was taken out of the plasma using 3.5-kDa D-tube dialyzers (EMD Millipore) in PBS (Villeda et al. 2014).

DNA isolation

Genomic DNA isolated from cecum content "Quick DNA TM Fecal/Soil Microbe Miniprep Kit, Cat. No. D6010". The amount and purity of the isolated DNA were determined fluorometrically by Qubit.

Amplification of the 16 s rRNA V3–V4 region

V3–V4 regions of the 16 s rRNA gene to be used for species determination were amplified with universal 341F (CCT ACGGGNGGCWGCAG) and 805R (GACTACHVGGG TATCTAATCC) primer sequences using SimpliAmp Thermal Cycler.

PCR conditions of 16 s V3–V4 regions

PCR conditions: 95 °C 10 min, first denaturation (HS enzyme will be used), 35 cycles: 95 °C for 45 s—denaturation, 50–55 °C for 45 s—adhesion, 72 °C for 60 s, elongation, 72 °C for 3 min—final elongation, the temperature was reduced to 4 °C and PCR was completed (Ilıkkan and Bağdat 2021).

Library preparation and sequencing

Illumina's "Nextera XT DNA Library Prep Kit, Cat. No: FC-131-1096" was used to prepare the library for the 16 s rRNA V3–V4 amplicon products, and the "TG Nextera XT Index Kit v2 Set A (96 Indices, 384 Samples), Cat. No: TG-131-2001 was used to index the library. Beckman Coulter's "AMPure XP beads" were used for PCR purification. Illumina's Miseq technology was used to sequence the data as paired-end (PE) 2 × 150 base reads. Each sample must have a minimum of 30,000 readings (Dorado et al. 2019; Gurbanov et al. 2022).

Bioinformatics analysis of raw data

The raw sequence data (FastQ) was subjected to quality checks to increase the accuracy of microbial diversity estimation and to filter out sequencing artifacts such as low-quality reads and contaminated reads, followed by trimming using FastQC v0.10.1 if needed. The Kraken Metagenomic system was used to cluster the sequence data into OTU groups (Yang et al. 2015). Heatmaps were created with GraphPad Prism 8.0.1 (GraphPad Software, USA) software. All the sample raw reads have been deposited at NCBI under the BioProject ID PRJNA857892 (Wood and Salzberg 2014).

Shannon and Simpson's diversity indexes

For adiversity, Shannon diversity indices were determined at the species level. The value of Shannon's Equitability ranges between 1.5 and 3.5. The greater this number, the more even the distribution. The Simpson indexes were calculated using the abundance and uniformity of OTUs. Simpson's Diversity Index (1-D) is a numeric number between 0 and 1, where 1 equals total evenness (Kalamaki and Angelidis 2020).

Statistics

The statistical analyses were given as mean ± standard error of the mean (SEM). Unpaired *T* test (one-sided *p* value) was utilized to compare the alpha diversities and F/B ratio between plasma-recipient (*P*) and control (*K*) groups. The comparison was done in GraphPad Prism 8 (GraphPad Software, USA) software. The degree of significance was denoted as $*p < 0.05$ and $***p < 0.001$. Heatmap analysis of metagenomic counts for bacterial families, genera, and species in plasma-recipient (*P*) and control (*K*) groups was performed in GraphPad Prism 8 (GraphPad Software, USA) software.

Results and discussion

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. The 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). According to alpha diversity results; the Shannon index (*H*) value is increased in the gut microbiota of the *P* group (young plasma recipients, 310,610 read) compared with the *K* (control, 207,742 read) one (Fig. 1A).

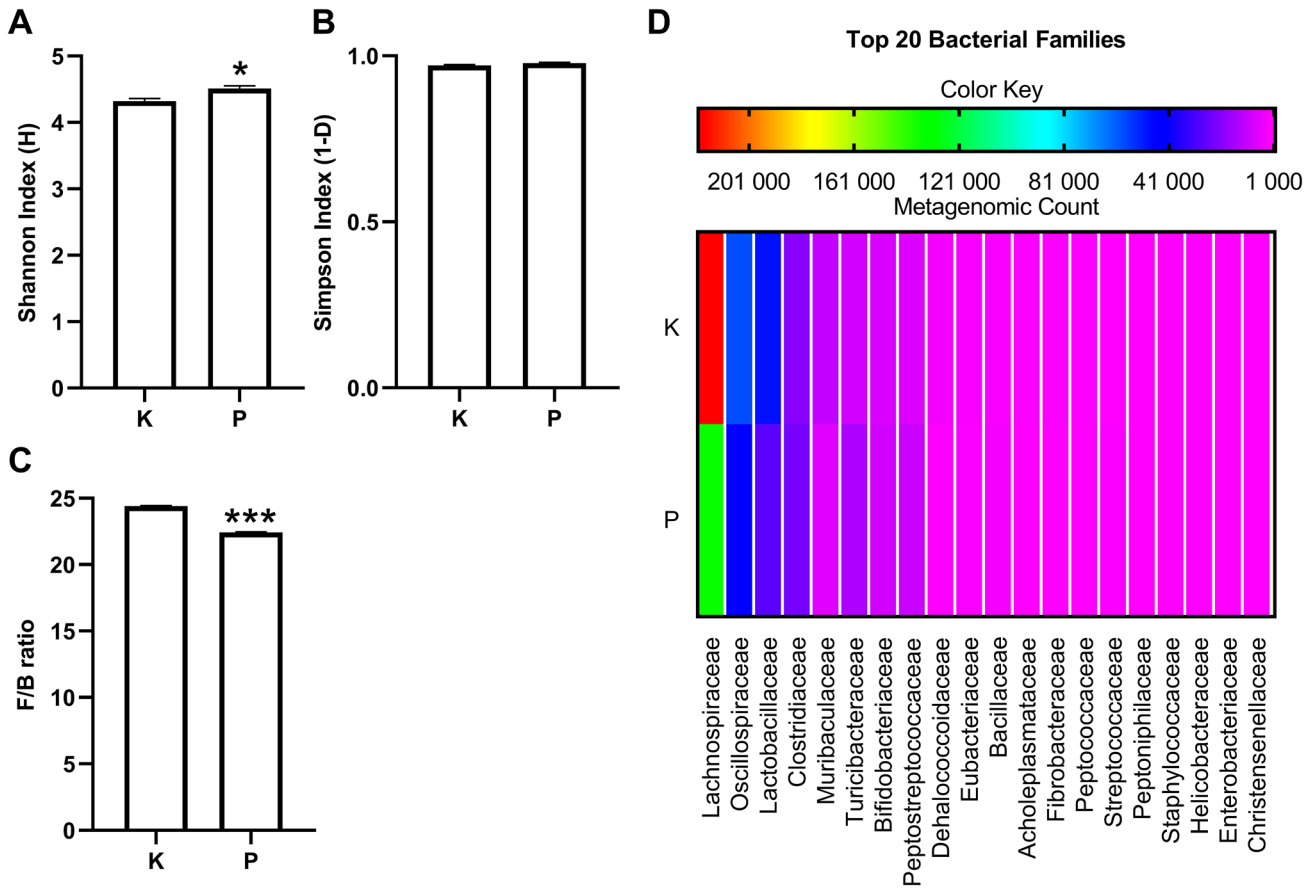

Fig. 1 Impact of young rat plasma on middle-aged rats' microbiota. **A** Shannon (H) and **B** Simpson (1-D) indices, **C** Firmicutes to Bacteroidetes ratio (F/B ratio), and **D** heatmap visualization of metagenomic counts of bacterial families (*p* values from top to bottom: < 0.0001, 0.0003, 0.0005, 0.3253, 0.0066, 0.0004, 0.0018, 0.0085, 0.0002, 0.0149, 0.028, 0.0001, 0.0006, 0.0035, 0,0137, 0.0044, 0.0355, 0.001, 0.054, 0.0057)

However, there was no significant change in another index for alpha diversity that is the Simpson index (1-D) (Fig. 1B). Therefore, our results can be interpreted as a larger bacterial richness rather than evenness in the *P* group. In other words, the number of different species in the *P* group microbiota was found to be larger than that of the *K* group. The Simpsons index takes a value between 0 and 1; hence the obtained values for both groups are highest (> 0.97). It can be read as an evenly distributed species, i.e., similarly high bacterial abundances in both microbiotas. Diminishment in alpha diversity was previously linked to cognitive deficits, reduced reaction times, and impaired verbal fluency in individuals over the age of 40 (Verdi et al. 2018).

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 (Yang et al. 2015; Arias et al. 2019; Magne et al. 2020). Previously, high F/B ratios were associated with a dysbiotic microbiome and it is reported to increase from birth to adulthood (Mariat et al. 2009; Indiani et al. 2018). Some other studies are linking the F/B ratio changes with senescence. However, it is still poorly understood whether the microbiota fluctuations can be associated with host aging or not (Kim and Benayoun 2020). Furthermore, high F/B ratios were also associated with Alzheimer's disease (AD) pathogenesis along with age in mouse studies (Seo and Holtzman 2020). In this study, the F/B ratio was significantly diminished in the P group microbiota, confirming possible rejuvenation properties of young plasma in middle-aged animals (Fig. 1C).

Dominant bacterial families are presented as heatmap visualizations across studied groups (Fig. 1D). When compared with control rat microbiota, the Lachnospiraceae, Oscillospiraceae, Lactobacillaceae, Muribaculaceae, and Dehalococcoidaceae families are the most diminished families in descending order, whereas the Clostridiaceae,

Turicibacteraceae, Bifidobacteriaceae, Peptostreptococcaceae, and Bacillaceae families are the most increased families in P group microbiota. It is obvious that plasma administration seriously affected the quantities of several dominant families in microbiota. However, for the explicit determination of plasma-induced fluctuations in microbiota, genus and especially species level examinations are more logical and descriptive.

Fluctuations in the microbiota in terms of dominant bacterial genera and species are also compared between both groups (Fig. 2). The diminished genera were *Anaerostipes, Butyrivibrio, Lactobacillus, Ruminococcus, Lachnoclostridium, Anaerotignum, Ligilactobacillus, Blautia, Roseburia, Herbinix, Flintibacter, Lacrimispora, Intestinimonas*, and *Duncaniella* in the P group. On the other hand, the increments in the counts of *Clostridium, Turicibacter, Bifidobacterium, Coprococcus*, and *Anaerocolumna* genera were estimated for the P group (Fig. 2A).

Because of the central role of the intestinal microbiota on the immune system, the strategies to strengthen the immune system senescence are critically dependent on the understanding of the aging process in the host–microbe consortium (Bosco and Noti 2021). Since chronological senescence cannot be reversed, it is important to comprehend healthy aging processes and the role of dynamic microbiota in this phenomenon to develop anti-aging therapies. However, the animal model, as well as human studies on the relationship between aging and longevity and microbiota, have revealed contradictory results in terms of specific bacteria taxonomies (Badal et al. 2020). Recently, a negative correlation has been reported between extreme aging and the abundance of *Coprococcus, Roseburia,* and *Faecalibacterium* genera from the Lachnospiraceae and Ruminococcaceae families in Chinese centenarians. Diminished *Bifidobacteria* units, which are known as lactate producers, have been reported in centenarians and elderly people (Ragonnaud and Biragyn

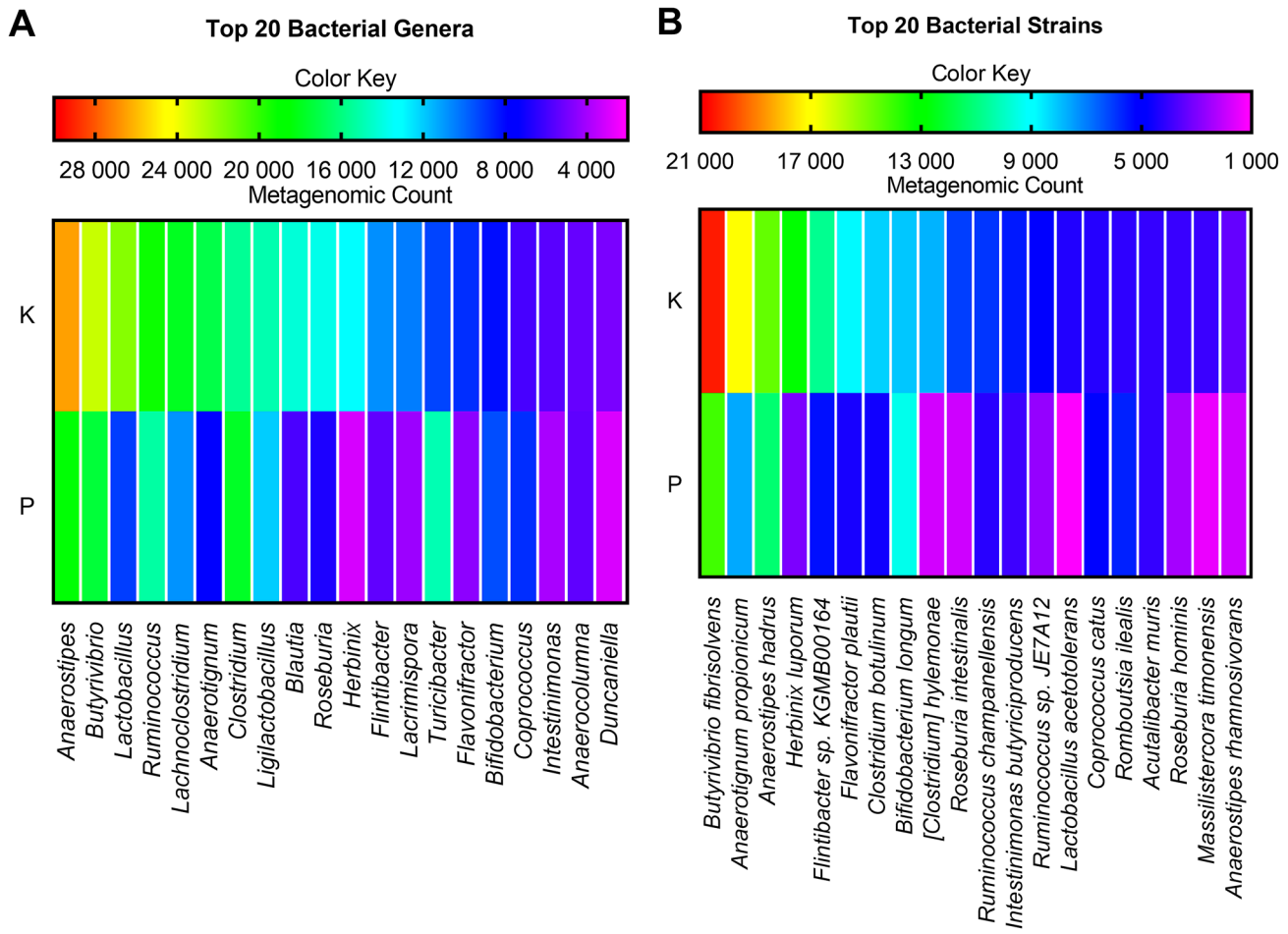

Fig. 2 Impact of young rat plasma on middle-aged rats' microbiota. Heatmaps of metagenomic counts of bacterial **A** genera (*p* values from top to bottom: < 0.0001, 0.0003, 0.0005, 0.3253, 0.0066, 0.0004, 0.0018, 0.0085, 0.0002, 0.0149, 0.028, 0.0001, 0.0006, 0.0355, 0.001, 0.054, 0.0057) and **B** species (*p* values from top to bottom: 0.0024, < 0.0001, 0.0033, 0.0003, 0.0042, 0.0015, 0.001, 0.0112, 0.8468, 0.0014, 0.0048, 0.0102, 0.001, 0.1572, 0.0258, 0.0189, 0.8243, 0.0432, 0.0053, 0,5033)

2021). Dysbiosis, or an imbalance in the microbial community structure, is a common accomplice of age-related disorders. In humans, age-related dysbiosis referred to as "microbe-aging" is characterized by a loss in Clostridiales and *Bifidobacterium* with enrichment in Proteobacteria and an overrepresentation of pathobionts such as Enterobacteriaceae (Bosco and Noti 2021). Aging was associated with the increased presence of specific Lactobacillus species, which were dominant in older adults (Badal et al. 2020).

In the case of species-level variations, *Butyrivibrio fibrisolvens, Anaerotignum propionicum, Anaerostipes hadrus, Herbinix luporum, Flintibacter* sp. KGMB00164*, Flavonifractor plautii, Clostridium botulinum, [Clostridium] hylemonae, Roseburia intestinalis, Ruminococcus champanellensis, Intestinimonas butyriciproducens, Ruminococcus* sp. JE7A12, *Lactobacillus acetotolerans, Roseburia hominis, Massilistercora timonensis,* and *Anaerostipes rhamnosivorans* species were depleted due to plasma administration. Increased counts of *Bifidobacterium longum, Coprococcus catus,* and *Romboutsia ilealis* species were observed in plasma-administered rats (Fig. 2B).

The well-known probiotics from the *Bifidobacterium* genus, such as *B. longum* (family: Bifidobacteriaceae), were shown to reverse impaired brain functions such as anxiety, uncoordinated movement, cognitive deficits, and hippocampus senescence, and can improve age-associated cognitive declines in d-gal-treated mice (Xia et al. 2020). To date, short-chain fatty acids (SCFAs) have been shown to be synthesized by more than 70 gut bacteria. SCFAs such as acetate, propionate, and butyrate were reported to be most important in human well-being, and Coprococcus catus (family: Lachnospiraceae) has been drawn particular attention among the species producing these molecules (Xu et al. 2020). Although little is known about the function of Romboutsia ilealis (Peptostreptococcacea family) in the gut, it has been predicted as a potential pathobiont/metabolism worsener associated with diabetes (Rodrigues et al. 2021) as well as a predisposition to obesity (Wei et al. 2020). On the other hand, it was reported that diminished counts of *Romboutsia* sp., is a potential biomarker of colorectal cancer associated with tumorigenic mucosa and adenomatous polyps (Mangifesta et al. 2018).

Conclusions

To the best of our knowledge, this is the first study demonstrating the impact of plasma treatment on the microbial ecology of gut microbiota at the animal level. Accordingly, plasma administration led to greater bacterial richness, i.e., an enhanced number of different species in the gut microbiota. Moreover, the F/B ratio was significantly diminished in plasma-receiving rats microbiota, confirming possible rejuvenation properties of young plasma. Furthermore, unique changes in various bacterial taxa of the microbiota have been described that could be valuable in future research on blood-based anti-aging treatments. Nonetheless, our findings suggest that even specific strains cannot be attributed solely with health-promoting and/or harmful characteristics, because different bacterial taxa in the gut interact in a contentious and unstable manner (Liu et al. 2020; Vacca et al. 2020). Instead, the singular taxonomic units with known and unknown properties can act dually in community level interactions, and it seems that our understanding of this complex ecology in the microbiota needs more detailed research. Future metagenomic research on reciprocal plasma exchange among young and aged (24 months) rats is being planned by our group for a comprehensive understanding of plasma administration on intestinal microbiota.

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Declarations

Conflict of interest The authors have declared that no competing interests exist.

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