Anti-Aging Effect of *Agrocybe aegerita* Polysaccharide through Regulation of Oxidative Stress and Gut Microbiota

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**Abstract:** Polysaccharides extracted from *Agrocybe aegerita* (AAPS) have various physiological effects. In this study, we used the naturally aging *Drosophila melanogaster* and D-galactose-induced aging mice as animal models to study the anti-aging effects of AAPS via the alleviation of oxidative stress and regulation of gut microbiota. Results showed that AAPS could significantly prolong lifespan and alleviate oxidative stress induced by H$_2$O$_2$ of *Drosophila melanogaster*. In addition, AAPS significantly increased the activities of antioxidant enzymes in *Drosophila melanogaster* and mice, and reduced the content of MDA. Furthermore, AAPS reshaped the disordered intestinal flora, increased the abundance ratio of Firmicutes to Bacteroidetes, and increased the abundance of beneficial bacteria *Lactobacillus*. Our results demonstrated that AAPS had good antioxidant and potential anti-aging effects in vivo.

**Keywords:** *Agrocybe aegerita* polysaccharides; anti-aging; antioxidant activity; gut microbiota

1. Introduction

Aging refers to the inevitable degenerative changes of organ functions, eventually leading to disease and death. The aging process is affected by genetic and environmental factors, and the specific aging mechanism is still unclear. The free radical theory is more established in the field of aging research [1]. In 1956, Denham Harman first proposed the free radical theory of aging, arguing that the changes in cellular function during aging are caused by radical oxygen species accumulation, which can lead to biomolecular oxidation and cell damage [2]. Since then, this theory has been widely studied, recognized, and gradually developed into a relatively complete oxidative stress theory. This theory proposes that the synthesis rate and the activity of antioxidant enzymes and substances decline during aging. Thus, the oxidant metabolites accumulate, and the balance between the production and scavenging of free radicals is disrupted, resulting in the accumulation of free radicals. Excess free radicals can trigger lipid peroxidation, destroy the redox homeostasis of cells, cause irreversible tissue damage, and ultimately accelerate the aging process [3–5]. Therefore, studying the mechanisms related to aging and anti-oxidation, and exploring natural food with antioxidant activity are essential for addressing aging-related problems.

D-galactose (D-Gal) is a reducing aldohexose. In healthy adults, the galactose from a regular diet can be metabolized into glucose and absorbed by the body. However, long-term and high-dose injections of D-Gal into animals can promote galactose conversion to aldose and hydrogen peroxide under galactose oxidase, which then leads to the accumulation of intracellular reactive oxygen species [6]. The increased reactive oxygen species can cause...
oxidative stress, inflammation, mitochondrial dysfunction, and apoptosis, all of which contribute to body aging [7]. In addition, *Drosophila melanogaster* has a short life cycle and strong reproductive ability, and it is easy to feed and distinguish genders; moreover, *Drosophila* has very similar pathogenic and aging genes to humans [8]. Therefore, the D-gal-induced aging model in mice and *Drosophila* are widely used to screen antioxidants and anti-aging drugs [9].

*Agyrocybe aegerita* is a well-known edible mushroom that grows in northern temperate and subtropical regions, which is popular among people due to its pleasant smell and taste. It has high nutritional value and contains various active ingredients, including polysaccharides, leucine, glutamate and essential amino acid [10]. Accumulating studies show that *A. aegerita* has many physiological functions, e.g., as an antioxidant [11], anti-virus [12], anti-angiogenic [13], and for treating thrombosis [14]. The *A. aegerita* polysaccharide (AAPS) is one of the main active components in its fruit body. Our previous studies have found that AAPS possesses potent anti-aging activity in vitro, representing increased cell viability and decreased mitochondrial membrane potential [15]. It has also been shown to be a suitable prebiotic combined with *lactobacillus rhamnousus* GG alleviating oxidative stress [16]. However, whether AAPS possesses anti-aging effects via regulation of oxidative stress and gut microbiota has not yet been explored in vivo.

In this study, we investigated whether AAPS has an anti-aging effect via alleviating oxidative stress and regulation of gut microbiota. For this purpose, the protective effects of AAPS against natural aging and H₂O₂-induced oxidative stress in the *Drosophila* model were evaluated. Moreover, the possibility that AAPS treatments could enhance the antioxidant enzyme activities in *Drosophila* and aging mice was explored. In addition, the gut microbiota in mice was examined by high-throughput sequencing.

2. Materials and Methods

2.1. Chemicals and Materials

*A. aegerita* (3MTJ-09106) was purchased from Gutian Tianxian Agricultural Products Co., Ltd. (Ningde, China). AAPS was prepared from *A. aegerita* according to our previous works [15]. Lentinan (LE) (Z20080579) was purchased from Hubei Chuangli Pharmaceutical Co., Ltd. (Hubei, China). The kits for estimating total superoxide dismutase (T-SOD) (A001-1-2), total antioxidant capacity (T-AOC) (A015-2-1), glutathione peroxidase (GSH-Px) (A005-1-2), catalase (CAT) (A007-2-1), and malondialdehyde (MDA) (A003-1-1) were purchased from Nanjing Jiancheng Biochemical Co., Ltd. (Nanjing, China). All other chemicals and reagents used were of analytical grade and commercially available.

2.2. Fly Strains and Diet

Fly strains used in this experiment were wild-type *Drosophila melanogaster* Oregon (Fujian Agriculture and Forestry University, Fuzhou, China). A basal diet was prepared using the following methods: 31 g of sucrose and 3 g of agar were added to 300 mL of three-distilled water, heated and stirred until fully dissolved. Then, 42 g of corn flour was added to 160 mL of three-distilled water, mixed well, poured into a pot, stirred and boiled until the mixture was pasty. When the mixture was cooled down to room temperature, 3 g of yeast powder and 3 mL of propionic acid were added and stirred. Then 3 mg/mL LE, 3 mg/mL and 9 mg/mL AAPS were added as the experiment medium. The diets were aliquoted into sterilized culture tubes and prepared every three days. Flies were raised in an incubator at (24 ± 1) °C, 50–60% humidity, and 12 h of light/dark cycle.

2.3. Lifespan Assay

Lifespan assay was performed as previously described with slight modifications [17]. A total of 800 male and female flies that had not mated within 8 h of eclosion were collected and randomly divided into four groups (200 per group, half male and half female). The normal control group (NC) was fed the basal diet, and the other groups were fed 3 mg/mL Lentinan (LE), 3 mg/mL AAPS (AL) and 9 mg/mL AAPS (AH), respectively. The number
of dead fruit flies was recorded at a fixed time every day until all the fruit flies died. The average lifespan and longest lifespan of fruit flies were analyzed. The longest lifespan is the average lifespan of the last 10% of fruit flies in each group.

2.4. Hydrogen Peroxide (H$_2$O$_2$) Challenge Assay

Hydrogen peroxide (H$_2$O$_2$) challenge assay was performed as previously described with slight modifications [18]. H$_2$O$_2$ was applied to flies to trigger oxidative stress. After 20 days of feeding, a total of 800 male and female flies were collected, and randomly divided into four groups (200 per group, half male and half female), and transferred into new culture tubes containing filter paper saturated with 200 µL of 30% H$_2$O$_2$ in a 6% glucose solution. The number of dead flies was recorded every three hours until all flies died.

2.5. Animals and Treatment

Male Kunming mice, 4–6 weeks, were provided by the Experimental Animal Center of the Fujian Medical University, Fujian, China (quality certificate number: SCXK (Min) 2016-0002). All animals were raised and handled following the Guidelines for the Care and Use of Laboratory Animals. All animal experiments and procedures were approved by the Animal Ethics Committee of Fujian Medical University, and the approval number is 2017-0120.

They were housed at 22 ± 2 °C with a 12 h light-dark cycle and provided free access to food and water. After one week of adaptation, the mice were randomly assigned to 5 groups (n = 12): normal control group (NC), model control group with D-Gal (MC), 300 mg/kg.bw/day Lentinan group (LE), 300 mg/kg.bw/day AAPS group (AL), 600 mg/kg.bw/day AAPS group (AH). Except for the control group, all groups were subcutaneously injected with D-galactose dissolved in normal saline (0.9%, w/v) (120 mg/kg.bw) once daily for 8 weeks. Meanwhile, LE and AAPS groups were orally gavaged with LE or AAPS for 8 weeks; and the NC and MC groups received normal saline solution at the same volume. After the endpoint of the experiment, all animals were sacrificed and the serum, brain, and cecal contents were collected and stored until use.

2.6. Biochemical Analysis

Flies from each group were sacrificed by frozen, and homogenized with normal saline in the ice bath. The homogenate was then centrifuged at 3000 r/min for 10 min at 4 °C. The supernatant was collected to measure the protein content, T-SOD activity, CAT activity, GSH-Px activity and MDA content by corresponding kits.

The brain tissues of mice were homogenized and centrifuged, and the supernatant was collected. The supernatant and serum samples were used to measure the protein content, T-AOC activity, CAT activity, GSH-Px activity and MDA content by corresponding kits. All of the procedures completely complied with the manufacturer’s instructions.

2.7. Intestinal Microbiota Analysis

Genomic DNA was extracted from mice’s cecal content using the Qiagen DNeasy kit. DNA obtained from each sample was diluted to the same concentration (10 nmol/L) and detected by 1% agarose gel electrophoresis. The V3-V4 region of the bacterial 16S rDNA was amplified using a general primer to generate an amplicon of 480 bp. Illumina MiSeq-PE250 system was employed for the paired-end amplicon sequencing according to manufacturer’s recommendations. For the microbial diversity analysis, the data were analyzed using the Majorbio I-Sanger Cloud Platform (Shanghai, China).

2.8. Data Analysis

The experimental results were expressed as mean ± standard deviation (Mean ± SD). GraphPad Prism 8.0 and SPSS 19.0 were used for plotting and statistical processing. Log-rank test was used for the survival test to analyze the significance of life expectancy. D’Agostino & Pearson test was used to assess the normal distribution of data, and then
the data was treated in parametric way using Student’s t test. The mean difference was considered significant at \( p < 0.05 \) or \( p < 0.01 \).

3. Results
3.1. Effects of AAPS on the Lifespan of Drosophila

Aging is a natural process with highly complex progressions. Lifespan is the most direct observational index to evaluate the anti-aging effects in experiments. As shown in Table 1 and Figure 1, AAPS supplementation could extend the lifespan of both female and male fruit flies. Compared with the male NC group, the average lifespan of male fruit flies in the AH group was prolonged from 42.74 to 46.85 days (\( p < 0.05 \)), with a 9.62% increase. The average lifespan of female flies in the AL group was increased from 45.14 to 49.12 days (\( p < 0.05 \)). The longest lifespan of both female and male flies in each AAPS group was significantly prolonged (\( p < 0.05 \) or \( p < 0.01 \)). Compared with the NC group, the survival curves of AAPS groups were more right dispersed (Figure 1).

Table 1. Effects of Agrocybe aegerita polysaccharide on the lifespan of Drosophila.

| Group | Mean Lifespan (d) | Maximum Lifespan (d) | Median Survival (d) | Prolongation of Mean Life Span (%) |
|-------|-------------------|----------------------|---------------------|----------------------------------|
| Male  |                   |                      |                     |                                  |
| NC    | 42.74 ± 1.66      | 61.70 ± 0.38         | 50.5                |                                  |
| LE    | 47.00 ± 1.60 *    | 65.40 ± 0.32 **      | 53.5                | 9.97                            |
| AL    | 45.57 ± 1.56      | 62.80 ± 0.42 *       | 52.5                | 6.62                            |
| AH    | 46.85 ± 1.53 *    | 64.60 ± 0.47 **      | 54                  | 9.62                            |
| Female|                   |                      |                     |                                  |
| NC    | 45.14 ± 1.61      | 63.20 ± 0.24         | 51.5                |                                  |
| LE    | 47.91 ± 1.67      | 67.10 ± 0.33 **      | 55                  | 6.14                            |
| AL    | 49.12 ± 1.64 *    | 69.70 ± 0.66 **      | 56                  | 8.82                            |
| AH    | 48.79 ± 1.61      | 69.00 ± 0.60 **      | 54                  | 8.09                            |

Note: data are presented as the means ± SEM (\( n = 100 \) per group). * \( p < 0.05 \) vs. NC group. ** \( p < 0.01 \) vs. NC group.

Figure 1. Effects of AAPS on the lifespan of Drosophila melanogaster. (A) male and (B) female. LE, 3 mg/mL Lentinan; AL, 3 mg/mL AAPS; AH, 9 mg/mL AAPS; (\( n = 100 \) per group).

3.2. Effects of AAPS on H\(_2\)O\(_2\)-Induced Oxidative Stress in Drosophila

Kaplan-Meier survival curve analysis showed AAPS could alleviate oxidative stress caused by H\(_2\)O\(_2\) (Figure 2). Compared with the male NC group, the average lifespan in AL and AH groups were improved by 19.27% (\( p < 0.01 \)) and 14.41% (\( p < 0.01 \)), respectively (Table 2). The addition of AAPS also extended the lifespan of female groups. As the concentration of AAPS in 3 mg/mL and 9 mg/mL, compared with the control group, the mean lifespans were improved by 11.27% (\( p < 0.01 \)) and 15.94% (\( p < 0.01 \)), respectively. AAPS supplemented also positively extended the longest lifespan of both female and male flies (\( p < 0.01 \)).
Figure 2. Effects of AAPS on H$_2$O$_2$ treatment on the survival time of *Drosophila melanogaster*. (A) H$_2$O$_2$ treatment of male (B) H$_2$O$_2$ treatment of female. LE, 3 mg/mL Lentinan; AL, 3 mg/mL AAPS; AH, 9 mg/mL AAPS; (n = 100 per group).

Table 2. Effects of *Agrocybe aegerita* polysaccharide on the lifespan of H$_2$O$_2$ treated *Drosophila*.

| Group | Mean Lifespan (h) | Maximum Lifespan (h) | Median Survival (h) | Prolongation of Mean Life Span (%) |
|-------|-------------------|----------------------|---------------------|-----------------------------------|
| Male  |                   |                      |                     |                                   |
| NC    | 17.28 ± 0.48      | 24.60 ± 0.38         | 18                  | -                                 |
| LE    | 19.68 ± 0.52 **   | 27.60 ± 0.38 **      | 21                  | 13.89                             |
| AL    | 20.61 ± 0.53 **   | 29.10 ± 0.43 **      | 21                  | 19.27                             |
| AH    | 19.77 ± 0.53 **   | 28.50 ± 0.47 **      | 21                  | 14.41                             |
| Female|                   |                      |                     |                                   |
| NC    | 18.63 ± 0.57      | 28.80 ± 0.46         | 18                  | -                                 |
| LE    | 20.82 ± 0.61 **   | 30.90 ± 0.43 **      | 21                  | 11.76                             |
| AL    | 20.73 ± 0.61 **   | 31.50 ± 0.47 **      | 21                  | 11.27                             |
| AH    | 21.60 ± 0.63 **   | 30.60 ± 0.38 **      | 21                  | 15.94                             |

Note: data are presented as the means ± SEM (n = 100 per group). ** p < 0.01 vs. NC group.

3.3. Effects of AAPS on the Antioxidant Capacity of Drosophila

After 30 days of AAPS treatment, the content level of MDA and the enzyme activity of T-SOD, CAT, and GSH-Px related to oxidative stress capacity were measured in flies. As shown in Figure 3, the female AH group showed significantly increased T-SOD levels compared with the NC group, with a 7.10% increase (p < 0.05). Moreover, the high-AAPS treatment increased the CAT levels in male flies (p < 0.05), which was 6.88% higher than the NC group (p < 0.05). In addition, the activity of CAT in each AAPS group significantly increased compared with the NC group (p < 0.05 or p < 0.01). The levels of lipids oxidation end-product MDA in both male and female AH groups were remarkably lower than the NC group (p < 0.05).

3.4. Effects of AAPS on the Antioxidant Capacity of Mice

After 8 weeks of AAPS treatment, the AAPS-treated groups showed significant improvement in antioxidant activities (Figures 4 and 5). The antioxidant enzyme (T-AOC, CAT and GSH-Px) activities in serum and brain of the MC group were obviously lower than those of the NC group (all p < 0.01). Meanwhile, the antioxidant enzyme (T-AOC, CAT and GSH-Px) activities in the serum and brain of the AH group were significantly increased (p < 0.05) than those of the MC group. The level of T-AOC and GSH-Px in the serum and brain of the AL group were also significantly increased (p < 0.05). The level of MDA in the serum and brain of two AAPS-treated groups was significantly attenuated (p < 0.01). In contrast, those of the MC group were significantly increased compared with the NC group (p < 0.01).
Figure 3. Effects of AAPS on the antioxidant capacity of *Drosophila melanogaster* (*n* = 100 per group). (A) T-SOD. (B) CAT. (C) GSH-Px. (D) MDA. LE, 3 mg/mL Lentinan; AL, 3 mg/mL AAPS; AH, 9 mg/mL AAPS. Data are presented as the means ± SEM. * *p* < 0.05 vs. NC group. ** *p* < 0.01 vs. NC group.

Figure 4. Effects of AAPS on the antioxidant capacity in serum of mice (*n* = 10 per group). (A) T-AOC. (B) CAT. (C) GSH-Px. (D) MDA. LE, 300 mg/kg.bw/d Lentinan; AL, 300 mg/kg.bw/d AAPS; AH, 600mg/kg.bw/d AAPS. Data are presented as the means ± SEM. # *p* < 0.05, ## *p* < 0.01 vs. NC group. * *p* < 0.05, ** *p* < 0.01 vs. MC group.
Figure 5. Effects of AAPS on the antioxidant capacity in brain of mice (n = 10 per group). (A) T-AOC. (B) CAT. (C) GSH-Px. (D) MDA. LE, 300 mg/kg.bw/d Lentinan; AL, 300 mg/kg.bw/d AAPS; AH, 600mg/kg.bw/d AAPS. Data are presented as the means ± SEM. # p < 0.05, ## p < 0.01 vs. NC group. * p < 0.05, ** p < 0.01 vs. MC group.

3.5. Effects of AAPS on the Composition of the Gut Microbiota

Fecal samples were sequenced to assess the effect of AAPS on the gut microbiota structure after 8 weeks of feeding. Then, 16S rDNA raw gene sequences were obtained from 25 samples through Illumina Miseq sequencing. The average number of sequences from each sample was 51,145. As shown in Figure 6A, the average OTU level of the Chao index from AAPS treatment groups was higher than that of the MC group, although there was no significant difference. The community barplot analysis and community heatmap analysis of the microorganism at the phylum level were shown in Figure 6B,C. At the phylum level, the major taxonomic units include Firmicutes, Bacteroidetes, Epsilonbacteraeota, Deferribacteres and Proteobacteria (Figure 6B). The increased abundance of Firmicutes and reduced amount of Bacteroidetes and Proteobacteria in the AAPS treatment groups indicated an increase in beneficial bacteria and a reduction in detrimental bacteria, compared with the MC group. The ratio of Firmicutes to Bacteroidetes was also increased. By displaying the major phylotypes in the heatmap, we observed that samples of AAPS treatment groups were clustered with that of the NC group (Figure 6C), which indicated that AAPS reshaped the disordered intestinal flora induced by D-gal injection. The abundances of beneficial Lactobacillus in the intestine of the AAPS treatment groups were higher than that of the MC group (Figure 6D).
Figure 6. Microbial Alpha diversity and composition analysis of mice treated with different diets. (A) Alpha diversity analysis of Chao index of OTU level. (B) Relative abundance on the phylum level in barplot analysis. (C) Species abundance clustering heatmap on phylum level, the closer distance and the shorter branch length between the two samples suggest that the species composition and abundance of the two samples is more similar; the color gradient from blue to red indicates the relative abundance from low to high. (D) Species abundance clustering heatmap on genus level. LE, 300 mg/kg.bw/d Lentinan; AL, 300 mg/kg.bw/d AAPS; AH, 600mg/kg.bw/d AAPS.

The similarity of the microbial communities in the five groups of mice was analyzed by Non-metric Multidimensional Scaling (NMDS), based on Bray-Curtis distance metrics (Figure 7A). The scatter plot showed that the microbiota of the NC group and AAPS treatment groups were mixed, while the MC group was different from other groups. In addition, the Spearman correlation heatmap showed the potential correlations between the oxidative stress indicators and gut microbiota (Figure 7B). Epsilonbacteraeota exhibited a positive with the MDA level in the serum, and was negatively associated with the GSH-Px level in both serum and brain. Patescibacteria exhibited a negative correlation with the CAT lever in the brain. Actinobacteria abundance was positively correlated with the GSH-Px levels in brain, but was negatively correlated with the expression level of CAT in serum. Furthermore, Proteobacteria and Bacteroidetes exhibited a negative correlation with the T-AOC level in the brain, while Firmicutes was negatively correlated with the MDA level in the brain.
4. Discussion

Aging is the gradual decline of body’s physiological integrity and adaptability over time, resulting in the loss of tissue functions [19]. There are many theories about aging, but the mechanisms of its occurrence and development have not been fully elucidated. Among the proposed theories, oxidative stress, inflammatory aging, and intestinal microbiota imbalance are the relatively established theories of aging [20–22]. In this study, the AAPS supplement enhanced the antioxidant capacity of D-galactose-induced aging mice and changed the structure of intestinal microbiota; moreover, the increase in antioxidant levels was strongly correlated with the changes in the abundance of critical intestinal bacteria. During aging, the activity of free radical scavenging enzymes is reduced. The ability to scavenge free radicals is weakened, resulting in a considerable accumulation of free radicals, which in turn causes DNA mutation, protein function damage, cell structure and organelle function damage, and ultimately accelerates aging [23]. Therefore, improving the activity of antioxidant enzymes and reducing oxidative stress can delay aging.

The antioxidant system in the body, including the glutathione system and superoxide dismutase, is an essential part of maintaining the intracellular redox balance [24,25]. Thus, the degree of aging can be indicated by oxidative stress biomarkers. Total antioxidants are the sum of the body’s non-enzymatic and enzymatic system antioxidants, and T-AOC can comprehensively reflect the body’s antioxidant capacity. CAT is another antioxidant enzyme in the body, which can scavenge free radicals, thereby reducing the peroxide produced by the body [26]. MDA is the final product of lipid peroxidation induced by free radicals on unsaturated fatty acids. It is cross-linked with proteins, peptides, or lipid polymers to continuously accumulate senile pigments or lipofuscin, resulting in body aging [27,28]. Therefore, MDA content can objectively reflect free radicals’ level and lipid peroxidation degree. Our study showed that the effect of AAPS on the lifespan of Drosophila was related to the increase of antioxidant enzyme activity and the decrease of MDA concentration, suggesting a causal relationship between antioxidant enzyme activity and aging (Figure 3).

In addition, the MDA level of the aging mice treated with D-galactose was higher than that of the NC group, and others antioxidant enzyme level were lower, indicating that the aging body was in a state of oxidative stress. After supplementing with different concentrations of AAPS, the oxidative stress was significantly alleviated (Figures 4 and 5). Previous studies
have reported the antioxidant effects of AAPS, including the strong ability of free radicals scavenging in vitro and improving the activity of antioxidant enzymes in the body, which are consistent with the results of our study [11,15,16]. However, further research is needed to reveal the molecular mechanism of AAPS increasing the activity of antioxidant enzymes and delaying aging.

Although the structure of intestinal microbiota is affected by various factors such as country, region, dietary habits, gender, and disease, it is still considered to be one of the critical factors affecting longevity. Many studies have found that changes in the composition of the intestinal microbiota are closely related to host aging [29–31]. The diversity of microbiota is positively correlated with body health and negatively correlated with gut vulnerability in the elderly [32]. Biagi et al. and Kong et al. found that the extremely long-lived people in China and Italy had greater intestinal microbiota diversity than young adults [32,33]. In this study, the Alpha diversity results showed that the average OTU level of the Chao index from AAPS treatment groups was higher than that of the MC group, although there was no significant difference (Figure 6A).

The intestinal microbiota structure of the elderly is very different from that of the young people, with decreased abundance of Firmicutes and an increased abundance of Bacteroidetes [34]. Sagi et al. found that, compared with wild-type (SOD1+/+) mice, the mice lacking antioxidant enzyme SOD1 (SOD1−/−) exhibited altered ratios of Firmicutes to Bacteroidetes; moreover, the redox imbalance caused by SOD1 deletion also altered intestinal microbiota and its metabolites [35]. The ratio of Firmicutes to Bacteroidetes decreased significantly in the elderly, which is consistent with the aging model group in our study. Notably, AAPS also significantly increased the ratio (Figure 6B).

Lactobacilli are known as health-promoting bacteria, which have long been regarded as one of the most abundant microorganisms in the human gastrointestinal tract. They are also recognized as beneficial host-related groups in human and animal microbiota [36,37]. Li et al. [38] not only confirmed that Lactobacillus had good free radical scavenging ability in vitro, but also found that administration of Lactobacillus in vivo significantly improved the aging-related indicators, and reduced the D-galactose-induced hepatic oxidative stress by modulating the Nrf-2 signaling pathway. Redox imbalance in the body can cause oxidative damage and alter the intestinal microbiota. In our study, AAPS treatment had a more significant impact on the relative abundance of Lactobacillus compared with the MC group (Figure 6D). The abundance of Lactobacilli in the intestine of the MC group was lower than that of other groups, indicating that the aging body is in a state of microbiota imbalance. After intervening with different concentrations of AAPS, the serum MDA of the mice was significantly decreased and the antioxidant enzyme activities were significantly increased; in addition, the abundance of beneficial Lactobacilli in the intestine was increased. This result is consistent with the findings from Kong et al. [39], and the increase in the composition and abundance of Lactobacillus helps to relieve oxidative stress. Therefore, our results suggest that AAPS may exhibit antioxidant effects by regulating the composition and abundance of Lactobacillus in the intestinal microbiota of the aging body.

Furthermore, the correlation study on anti-aging indicators after AAPS treatment and dominant bacteria in intestinal microbiota found that, Epsilonbacteriota exhibited a positive with MDA level in serum, and was negatively associated with the GSH-Px level in both serum and brain. Moreover, Proteobacteria and Bacteroidetes exhibited a negative correlation with the T-AOC level in the brain. At the same time, Firmicutes was negatively correlated with the MDA level in the brain (Figure 7B). This result further demonstrated that AAPS might regulate the structure and abundance of intestinal microbiota, which further affects the oxidative stress of the body and delays aging.

5. Conclusions

Population aging is a significant issue faced by all countries in the world. During aging, the body’s physiological integrity and adaptability gradually decline, resulting in a gradual loss of tissue functions. Therefore, the enhancement of anti-oxidative stress
function and the improvement of intestinal microbiota has become one of the bases for anti-aging strategies. This study confirmed that *Agrocybe aegerita* polysaccharide could prolong the lifespan of *Drosophila melanogaster*, alleviate oxidative stress induced by *H*2*O*2, enhance the anti-oxidative stress ability of *Drosophila* and mice, and regulate the composition of intestinal microbiota in mice, suggesting that AAPS has good antioxidant and anti-aging functions. However, this study has not yet fully elucidated the mechanism of how changes in intestinal microbiota mediated by AAPS treatment regulate oxidative stress and aging-related signaling pathways. Therefore, it is necessary to further investigate the roles of intestinal microbiota and its metabolites in the antioxidant and anti-aging functions of AAPS from the perspective of molecular biology, which will help to reveal the anti-aging mechanism of AAPS comprehensively.

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