Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company’s public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Effects of phycocyanin on pulmonary and gut microbiota in a radiation-induced pulmonary fibrosis model

Wenjun Li a,d,1, Lina Lu b,1, Bin Liu c,*, Song Qin a,*

a Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, Yantai, PR China
b School of Chemical Engineering, Northwest Minzu University, Lanzhou, PR China
c School of Stomatology, Lanzhou University, Lanzhou, Gansu, PR China
d Center for Ocean Mega-Science, Chinese Academy of Sciences, Qingdao, PR China

ARTICLE INFO
Keywords:
Thorax irradiation
Pulmonary fibrosis
Microbiota
Phycocyanin

ABSTRACT
Objective: Radiation pneumonia and fibrosis are major clinical complications of radiotherapy for thoracic tumor patients, and may significantly reduce survival and quality of life. At present, no safe and effective radiation protection measures have been approved for clinical use. Phycocyanin, a protein responsible for photosynthesis from Spirulina, has been shown to have a variety of biological activities and to be beneficial for a variety of diseases, including pulmonary fibrosis. However, the preventive and protective effects of phycocyanin on radiation-induced pulmonary fibrosis have not been studied.

Design: X-ray single dose irradiation was used on the chest of mice to prepare a mouse model of pulmonary fibrosis, from which the effect of phycocyanin on pulmonary histopathologic change, pulmonary fibrosis, the microbiota in lung and gut, LPS, TNF-α, and IL-6 at different time after irradiation were evaluated.

Results: Phycocyanin alleviated the radiation-induced lung injury and reduced the level of inflammatory factors. Thorax irradiation led to the disorder in microbiota of the lung and gut. The variation trend of the diversity of the two tissues was opposite, but that of the microbiota composition was similar. The phycocyanin intervention regulated the composition of the lung and gut microbiota, transformed them into normal state, and reduced the level of LPS, which significantly reduced the abundance of inflammation-related bacteria, and increased the abundance of probiotics that produce short-chain fatty acids.

Conclusion: Phycocyanin could regulate the radiation-induced disorder in lung and gut microbiota of mice, and reduce the radiation-induced lung inflammation and fibrosis.

1. Introduction
Radiation therapy for esophageal cancer, lung cancer, or other thoracic spine cancer often causes acute radiation pneumonia or chronic radiation pneumonia. The radiation-induced lung injury (RILI) can even further lead to lung fibrosis. The incidence of RILI in patients receiving thoracic radiotherapy and lung cancer patients receiving high-dose radiotherapy can reach 30%–40%, respectively [1,2]. Patients with RILI often present with cough, fever and chest pain. The consequences of RILI can even lead to respiratory failure and even death of patients. As it involves a variety of cells, the mechanism of RILI causing pulmonary fibrosis is more complicated, including complex intermolecular interactions. Radiation can cause apoptosis of epithelial cells, as well as a large amount of oxidative damage and the release of fibrotic cytokines. These processes eventually lead to excessive deposition of extracellular matrix ECM and the formation of lung fibrosis. In recent years, with the in-depth research on lung diseases, more and more studies have proved that there is an important crosstalk between gut microbes and the lungs, which plays an important role in maintaining the health of the host. Studies have shown that gut microbes can affect lung immunity, and many lung diseases are often accompanied by changes in gut microbes [3–5]. However, reports on the changes of lung microbiota in the lung and gut after RILI. In addition, facing the global pandemic of Covid-19, it is urgently needed to find active substances that can slow down the pneumonia and enteritis. Covid-19 can causes serious inflammation of human lungs, causing difficulty in breathing and pulmonary fibrosis,
and eventually lung failure. ACE2 in lungs and gastrointestinal tract may be the transmission route of Covid-19.

Phycocyanin (PC) is a light-harvesting pigment protein isolated from cyanobacteria and can participate in the photosynthesis of algae. In the past 20 years, the role of PC in promoting health and treating diseases has been widely studied and reported. Previous studies have shown that PC has multiple activities such as antioxidant and anti-inflammatory [6, 7]. It has been reported that PC can alleviate paraquat-induced lung injury in rats [8]. Research found that in the bleomycin-induced mouse lung fibrosis model, PC could regulate TLR2-MyD88-NF-κB signaling pathways alleviate pulmonary fibrosis [9], and in addition, could significantly reduce bacteria that associated with inflammation, thereby alleviating pulmonary fibrosis [10-12].

Thus, we hypothesized that, before or after RILI, PC intervention could change the colonizing pattern of the gut microbiota, thereby alleviating the lung injury. Therefore, we induced pulmonary fibrosis in mice by X-ray single-dose irradiation on thorax and studied the effect of PC on radiation induced pulmonary fibrosis by evaluating the changes in lung histopathology, inflammatory factor levels, and microbiota in the lung and gut.

2. Materials and methods

2.1. Ethics statement

Our experimental protocol was formulated in accordance with the 'Laboratory Animal Care Guidelines'. The animal experiment ethics committee of Lanzhou University approved the experimental protocol.

2.2. Materials and animals

PC was extracted from *Spirulina.* King Dnarmse Spirulina Company (Fuqing, China) provided a medical grade PC for this experiment. Male C57BL/6 mice were purchased from Lanzhou Medical College in China. All animals were raised at 22±2 °C and were given standard food and water.

2.3. Experimental design

4 to 6-week-old and male C57BL/6 mice were randomly divided into 4 groups in body weight, 15 mice in each group, i.e., the normal control group (C), radiotherapy only (RT), PC pre-administration plus radiotherapy group (PC + RT group), and radiotherapy plus PC administration group (RT + PC group). RT group received chest RT alone. PC + RT group received RT after 30 days with PC (50 mg/kg/d), no further administration after irradiation, only the same volume of double distilled water was given. RT + PC group was treated with PC (50 mg/kg/d) after RT. Animals in groups C and RT were given equal volume of distilled water. At the time points of 1, 3, and 5 months after RT, five animals in each group were given general anesthesia, blood samples, intestine, and lung tissues were collected. The RT conditions were as follows. All mice (C group, RT group, PC + RT group and RT + PC group) were under general anesthesia. Part of the mice (RT group, PC + RT group and RT + PC group) were irradiated to the entire chest by the X-ray therapy instrument (20 Gy, 2 Gv/min).

2.4. Sample collection

One month after the mice were irradiated, the feces from the cecum of the animals were collected and stored in liquid nitrogen at –80 °C for detection of bacterial 16S ribosomal RNA (rRNA) V3 and V4. At 1, 3 and 5 months after the mice were irradiated, 5 mice from each experimental group were selected and sacrificed. Animal serum, intestinal tissue and lung tissue were collected and stored in a refrigerator at –80 °C. The lung tissue was stained with 4 % hematoxylin-eosin (HE) and MASSON.

2.5. Histopathological analysis

The tissue sections were stained with HE and Masson and three C57BL/6 mice were used in each group. A Panoramic MIDI scanner was used to take pictures of the stained sections. Lung injury scores were assessed according to previous methods. In brief, a score of 0, 1, 2, 3 and 4 represents no damage, mild damage, moderate damage, severe damage and very severe histologic changes, respectively [55]. In addition, fibrosis was quantified using the modified Ashcroft scoring [13].

2.6. Lung and intestinal microbiota analysis

Wash the lungs of the mice with 0.5 mL of phosphate buffer. The lavage fluid is then collected and used for genetic testing [12, 14]. The extraction of total DNA and the evaluation of bacterial diversity used PowerSoil DNA isolation kit (MoBio Laboratories, Carlsbad, USA, California) and Illumina HiSeq platform.

2.7. Enzyme-linked immunosorbent assay (ELISA)

ELISA kits (Shanghai Enzyme Link Biotechnology Co., Ltd., China) were used to detect the contents of interleukin (IL)-6, lipopolysaccharide (LPS) and tumor necrosis factor alpha (TNF-α) in the samples.

2.8. Statistical analysis

Flora analysis used Wilcoxon rank sum test to determine the differences among groups. We use SPSS 19.0 software to analyze the results. If P < 0.05, the result was considered statistically significant.

3. Results

3.1. PC intervention reduced lung tissue damage and fibrosis caused by thoracic irradiation

During the experiment, we found that after irradiation at the chest cavity, the fur around the mouse’s chest was shed and turned pale with time. One month after irradiation, the fur was basically the same as that of the normal control group. However, after 3 and 5 months after irradiation, the fur was obviously whitened. From the appearance of fur, the whitening phenomenon of the PC intervention group was significantly less than that of the irradiation group (Fig. 1).

HE staining and Masson staining were used to analyze lung injury and fibrosis, and the results were compared 1 month after thoracic irradiation with that of group C, showing that the mouse lung tissue had obvious lymphocyte and neutrophil infiltration (green arrow in Fig. 2), alveolar tissue edema, and a small amount of blue collagen fibers were deposited. Three to 5 months after thoracic irradiation, with the prolongation of time, the lung tissue damage in mice increased, inflammatory cell infiltration increased, and eosinophilic mucus secretion appeared in the bronchial cavity (yellow arrow in Fig. 2). There is epithelial cell shedding in the tracheal cavity (blue arrow in Fig. 2), edema around the blood vessels, and loose connective tissue (red arrow Fig. 2). The alveolar wall thickens, collagen fibers proliferate, and fibroblasts increase (black arrows Fig. 2). Both prophylactic and therapeutic administration of PC reduced the infiltration of inflammatory cells and reduced the deposition of collagen fibers. This shows that PC intervention can alleviate lung injury caused by chest irradiation.

3.2. PC intervention reduces the increase of inflammatory factors and LPS induced by thoracic irradiation

Using the ELISA method, we detected the content of TNF-α, LPS and IL-6 in lung tissue, intestine, and serum. The results show that, the content of TNF-α and IL-6 in lung tissue or serum increased significantly at the three time points (1, 3, and 5 months) after thoracic irradiation.
compared with the normal control group, while the PC medication group the contents of TNF-α and IL-6 were decreased. Although compared with lung tissue and serum, the contents of IL-6 and TNF-α in intestinal tissue did not change significantly, but at the 5-month time point, the content of IL-6 and TNF-α in intestinal tissue was significantly higher than that of normal control group (Fig. 3a–b). Thoracic irradiation significantly increased the content of LPS in lung tissue, serum, and feces, and PC intervention also reduced its content to varying degrees (Fig. 3c).

3.3. PC intervention regulates lung and intestinal flora

We measured the lung and intestinal flora using 16sRNA technology. The results of α diversity analysis show that the diversity index of lung flora increased after irradiation compared to the normal control group. In addition, although the diversity of intestinal flora decreased but not as significantly as the lung flora did, the trend was opposite. Both pre-administration and therapeutic administration have reduced the change of the flora diversity to a certain extent (Fig. 3c), which showed that PC had a certain ability to regulate the disturbance of flora caused by thoracic irradiation.

After principal component analysis of each group, we found that no matter how the lung flora or intestinal flora varied, the difference in flora between groups C and RT was remarkable, and the bacterial structure after PC intervention was similar to that of group C (Fig. 5). In terms of the regulation of lung flora, the flora was more similar to that of group C. For the regulation of intestinal flora, the flora structure of PC + RT group was closer to that of group C (Figs. 4–6).

After analyzing the flora composition of each group, we found that at portal level, the main bacteria of normal lung flora are Fimicutes, Bacteroidetes, and Proteobacteria. Meanwhile, the intestinal flora included mainly Bacteroidetes and Fimicutes. Before irradiation, the proportion of Bacteroidetes doubled that of the original, while the content of Fimicutes decreased by about 15%. Although the change of intestinal flora was not as great as that of abdominal flora, the variation trends of Bacteroidetes and Fimicutes were the same to that of the lung flora for showing also the increase of Bacteroidetes and the decrease of Fimicutes. The PC intervention stabilized the normal flora to a certain extent. At the same time, in the PC-administered group, the content of Actinobacteria increased in both lung and intestinal flora (Fig. 6).

Additional analysis of the flora composition at genus level showed that the abundances of *Alisipes*, *Lachnoclostridium* and *Bacteroides* in the
lung flora increased after irradiation, while those of Lactococcus, Dubosiella, Lactobacillus, Turicibacter, Candidatus-Saccharimonas, Romboutsia, and Bifidobacterium decreased. In the intestinal flora after irradiation, the contents of Alisipes, Mucispirillum, Helicobacter, Turibacter, Parabacteroides, Lachnoclostridium, and Intestinimonas increased, while those of Alloprevotella, Muribaculum, Anaerotruncus, Enterococcus, Bacteroides, Ruminiclostridium, Lactococcus, and Lactobacillus decreased. After irradiation, five genera of Alisipes, Lactococcus, Lactobacillus, Lachnoclostridium, and Bifidobacterium showed the same trend in the lungs and intestines. However, PC administration alleviated the changes of genera such as Alisipes, Lachnoclostridium, Alistipes, Lactobacillus, Mucispirillum, Helicobacter, Turibacter, Parabacteroides, Intestinimonas, Bifidobacterium etc. Although the Faecalibacterium in the lungs and intestines did not change significantly after irradiation, PC intervention increased the relative abundance of Faecalibacterium in the lungs and intestines (Fig. 7).

The Spearman method was used to analyze the correlation between the levels of factors (TNF-α, LPS and IL-6) in the sample and the abundance of different bacterial species. The results show that Lachnoclostridium was positively correlated with the above indicators in the lung flora; Lactococcus, Dubosiella, Lactobacillus, Turicibacter, Candidatus-Saccharimonas, Romboutsia, and Bifidobacterium were negatively correlated with the above indicators, and the correlation was statistically significant (Fig. 8a). In the intestinal flora, Alistipes, Parabacteroides, Parasutterella, Lachnoclostridium, and Intestinimonas were positively correlated with the above indicators; while Bifidobacterium, Lactococcus, and Lactobacillus were negatively correlated with the above indicators (Fig. 8b).

4. Discussion

In this paper, we studied the preventive and protective effects of PC on radiation-induced lung injury, and analyzed its regulatory effects on radiation-induced disturbances of lung and intestinal flora. The results show that the whitening phenomenon of the PC intervention group was significantly less than that of the irradiation group and both PC pre-administration and therapeutic administration reduced the levels of inflammatory factors and LPS in lung tissue, serum, and intestinal tract. In addition, chest irradiation can lead to disorders of the lung and intestinal flora, while PC intervention significantly alleviated the disorder of the flora. This result is very similar to our previous report on the effects of phycocyanin on bleomycin-induced pulmonary fibrosis and the intestinal microbiota in C57BL/6 mice [10].

Radiation-induced pulmonary fibrosis is a common and serious side effect of radiotherapy, which is usually inevitable and seriously affects the quality of life and survival rate of patients [15]. According to the different irradiation time, radiation pulmonary fibrosis includes early inflammation and late fibrosis [16]. In the experiment, we used HE and Masson staining methods to analyze the degree of lung tissue damage and fibrosis. After irradiating the chest cavity with a dose of 20 Gy, we...
found that the structure of the alveoli showed varying degrees of damage and pulmonary fibrosis. Both pre-administration and therapeutic administration of PC reduced inflammation damage and collagen fiber deposition, which indicates that PC can relieve fibrosis to a certain extent.

A large number of early inflammatory factors can directly damage...
lung cells and further increase the permeability of pulmonary vascular endothelial cells and alveolar epithelium, leading to pulmonary edema and thrombosis. These processes eventually cause pulmonary fibrosis. The release of pro-inflammatory cytokines is related to various signaling pathways, such as the activation of NF-κB. If the expression of inflammatory cytokines can be reduced, the progression of pulmonary fibrosis can be reduced. Studies have shown that the progression of pulmonary fibrosis is related to the ongoing inflammation, mainly related to increased interleukins and tumor necrosis factor [17,18].

In this study, PC intervention significantly reduced the levels of TNF-α, LPS and IL-6, in the lung tissue, intestine, and blood of a mouse model with lung injury induced by chest irradiation. This indicates that the
anti-pulmonary fibrosis effect of PC may be related to the reduction of inflammation and lipopolysaccharide levels. It has been shown that in allergic inflammation, PC can significantly inhibit the release of TNF-α, IL-6, IL-1β, and neutrophil infiltration. PC can reduce the increase of TNF-α, IL-1β, and IL-6 caused by LPS-induced microglia [19]. In addition, PC can also have an important protective effect on inflammatory bowel diseases and macrophage activation [20]. These results indicate that PC can inhibit the release of inflammatory factors and LPS levels. In allergic inflammation, PC can significantly inhibit the release of inflammatory factors and the infiltration of neutrophils [19,20]. These results indicate that PC can reduce lung tissue damage caused by chest irradiation by inhibiting inflammation.

By analyzing the correlation between the flora and the physiological indicators of pulmonary fibrosis, we determined the importance of PC in regulating the flora. First, a large number of studies have shown that a variety of flora is involved in inflammation [21–23]. For example, Helicobacter can induce various cytokines and chemokines, such as TNF-α and IL-6 [24]. Helicobacter can produce ammonia and other harmful substances, causing damage to the intestinal wall. Some substances such as phospholipase and cytotoxin produced by Helicobacter can destroy the tight junctions of epithelial cells and cause apoptosis [25]. Helicobacter can cause inflammatory bowel disease in immuno-deficient mice [26], and is associated with a variety of enteritis diseases [27]. Alistipes were shown capable of inducing inflammation, and their abundance was positively correlated with blood levels of IL-6 and LPS [28,29]. Other bacteria related to inflammation are Parabacteroides and Bacteroides. Studies have shown that they were related to chronic inflammatory bowel disease and are more abundant in chronic inflammatory bowel disease [30,31]. Moreover, Intestinimonas could increase in abundance in animal models of inflammation [14]. Mucispirillum showed high abundance in the intestinal tract with oxidative stress and inflammation, which was also a sign of serious indigestion [32,33]. After thoracic irradiation, Helicobacter, Alistipes, Parabacteroides, Bacteroides, Intestinimonas, and Mucispirillum in the intestine proliferated in large quantities.

PC intervention significantly reduced the relative abundance of these strains and reduced the inflammatory damage of the bacteria to the tissue.

Secondly, a large number of studies show that many bacteria are involved in the production of short-chain fatty acids (SCFAs), such as Faecalibacterium, Ruminoclostridium, Marburgbaculum, Anaerotruncus, Romboutsia, Turicibacter, and Clostridiales [34–38]. They are beneficial bacteria, so they can ferment and produce SCFA in the intestine. SCFAs can reduce the level of intestinal inflammation, thereby protecting the intestinal barrier [39]. SCFAs can inhibit histone deacetylase and G protein coupled receptor (GPCR), while GPCRs are involved in lung diseases [40]. Compared with the irradiated group, the therapeutic administration of PC significantly increased the relative abundance of Faecalibacterium, Lachnoclostridium, Ruminoclostridium, and Clostridiales, increased the synthesis of SCFAs, and enhanced its inhibitory effect on HDACs and GPCRs.

In addition, many microorganisms are probiotics, such as Lactobacillus, Lactococcus, and Bifidobacterium [41]. Probiotics can improve the immunity of human mucosa by increasing immunoglobulins, and play an important role in human health [42]. Probiotics can regulate the balance of intestinal flora in the body by inhibiting the increase of pathogenic bacteria, which can help treat diseases [43]. For example, Lactobacillus interacts with many pathogens by secreting salivary hormone B (an antibiotic) [44]. Other studies have found that the fermentation of lactic acid bacteria can not only enhance the physiological activity of intestinal peristalsis, but also improve immunity and avoid hyperglycemia [45,46]. Bifidobacterium is also a beneficial bacteria in the intestinal tract, and it has immuno-modulation and hypoglycemic effects [47]. In this study, the relative abundance of Lactobacillus, Lactococcus, and Bifidobacterium increased significantly after PC intervention compared with the irradiated group alone, improving the immunity and balance ability of the flora.

Dysfunctional flora promotes the production of lipopolysaccharide (LPS). By binding to Toll-like receptor 4 (TLR4), LPS induces chronic low-grade inflammation [48]. LPS could not only directly damage tissues, but also cause systemic inflammatory cascades through blood circulation, leading to the release of inflammatory cytokines [49]. In this study, LPS levels were increased in lung tissue, blood, and feces after...
irradiation, while PC intervention reduced its levels in various tissues. In the correlation analysis, we also found that a variety of bacteria was significantly associated with LPS.

When analyzing the changes in the composition of the lung flora and intestinal flora, we found that there were many similarities between the changes in the lung flora and intestinal flora regardless of whether they were analyzed on the portal level or the genus level. For example, the abundance of *Firmicutes* was decreased after irradiation, and those of *Bacteroidetes* and *Actinobacteria* were increased after PC intervention. A previous study found that *Firmicutes* abundance was inversely related to the levels of various inflammatory factors, while *Bacteroidetes* was just the opposite [50]. This shows that the inflammation level of lung tissue is closely related to the flora of the lungs and even the intestine. Specific to the genus level, irradiation simultaneously reduced the abundance of *Lactobacillus*, *Lactococcus*, and *Bifidobacterium* in the lungs and intestines. After PC intervention, the abundance of the three bacteria in the lungs and intestinal flora were also increased. In addition, the experimental results of the PC + RT group and the RT + PC group show that both preventive and therapeutic intervention of PC can alleviate the pulmonary fibrosis, but there are some differences in regulating the composition of the flora. This may be due to the PC intervention promoted the colonization of beneficial bacteria after the mice in the PC + RT group took PC continuously for one month before irradiation, which may increase the tissue tolerance to irradiation. On the other hand, mice in the RT + PC group were irradiated before taking PC. Since irradiation itself can affect the composition of the mouse flora of the RT group, and PC intervenes on the basis that the flora had been affected by radiation, the degree of repair was different from the effect of PC preventive administration.

In 2020, Covid-19 has become an infectious disease worldwide. Lymphopenia and inflammatory cytokine storms are typical syndrome observed in Covid-19 patients and are believed to be related to the severity of the disease. Covid-19 can also cause severe inflammation of the lungs, causing difficulty in breathing and pulmonary fibrosis, and eventually the lung failure [51,52]. The Covid-19 virus attacks our body through angiotensin converting enzyme 2 (ACE2). ACE2 exists not only in our lungs, but also in our gastrointestinal tract. This may be the cause of gastrointestinal symptoms such as diarrhea, nausea, and vomiting in a large number of the patients. The gastrointestinal tract may be the potential transmission route and target organ of Covid-19 [53,54].

Therefore, there is an urgent need to find a natural active substance that can not only slow down the fibrosis caused by lung inflammation, but also adjust the structure of intestinal flora and maintain intestinal health. The findings of this study may provide a reference in this regard for the treatment of Covid-19 patients.

5. Conclusions

In summary, this study established a model of pulmonary fibrosis in thoracic irradiated mice, and evaluated the preventive and protective effects of PC. We believe that there was a communication mechanism between the intestine and lungs. HE and Masson staining results showed that PC significantly attenuated pulmonary fibrosis caused by radiation, reduced levels of IL-6, TNF-α, and LPS in the lung, serum, and intestine, which indicate that PC significantly reduced the levels of proinflammatory cytokines and lipopolysaccharide. PC intervention can obviously remedy the microbial disorder induced by irradiation, and the correlation between the biochemical indexes related to pulmonary fibrosis and the flora reveals that lung injury is closely related to the composition of the flora, and that there is communication between the intestine and the lungs. We infer that PC may play a protective role against radiation-induced lung injury by regulating the intestinal and lung flora, thus reducing LPS content and inflammatory response (Fig. 9). However, the mechanism of the interaction with the intestinal flora needs to be further explored.

---

References

[1] S.M. Bentzen, Preventing or reducing late side effects of radiation therapy: radiobiology meets molecular pathology, Nat. Rev. Cancer 6 (2006) 702–713.
[2] P.R. Graves, F. Siddiqui, M.S. Ancher, et al., Radiation pulmonary toxicity: from mechanisms to management, Semin. Radiat. Oncol. 20 (2010) 201–207.
[3] N.D.J. Ubags, B.J. Marsland, Mechanistic insight into the function of the microbiome in lung diseases, Eur. Respir. J. 50 (2017), 1602467.
[4] D.R. Samuelson, D.A. Welsh, J.E. Shellito, Regulation of lung immunity and host defense by the intestinal microbiota, Front. Microbiol. 6 (2015) 1085.
[5] Y. He, Q. Wen, F. Yao, et al., Gut-lung axis: the microbial contributions and clinical implications, Crit. Rev. Microbiol. 43 (2017) 81–95.
[6] W. Li, H. Su, Y. Pu, et al., Phycobiliproteins: Molecular structure, production, applications, and prospects, Biotechnol. Adv. 37 (2019) 340–353.
[7] C. Chang, Y. Yang, Y. Liang, et al., A novel phycobiliprotein alleviates allergic airway inflammation by modulating immune responses, Am. J. Respir. Crit. Care 183 (2011) 15–25.
[8] Y. Sun, J. Zhang, Y. Yan, et al., The protective effect of C-phycocyanin on paraquat-induced acute lung injury in rats, Environ. Toxicol. Pharm. 32 (2011) 168–174.
[9] C. Li, Y. Yu, W. Li, et al., Phycocyanin attenuates pulmonary fibrosis via the TLR2-MylD88-NF-κB signaling pathway, Sci. Rep.-UK 7 (2017) 5812–5843.
[10] Y. Xie, W. Li, C. Lu, et al., The effects of phycocyanin on bleomycin-induced pulmonary fibrosis and the intestinal microbiota in C57BL/6 mice, Appl. Microbiol. Biotechnol. 103 (2019) 8559–8569.
[11] Y. Xie, W. Li, Z. Zhu, et al., Effects of phycocyanin in modulating the intestinal microbiota of mice, Microbiologypogen 8 (2019) e825.
[12] L. Lu, W. Li, C. Sun, et al., Phycocyanin ameliorates radiation-induced acute intestinal toxicity by regulating the effect of the gut microbiota on the TLR4/MylD88/NF-kappal pathway, JPNJ. Parenter. Enteral Nutr. (2019).
[13] R. Hübner, W. Gitter, N. Eddine El Mokhtari, et al., Standardized quantification of pulmonary fibrosis in histological samples, Biotechnoliques 44 (2008) 507–517.
[14] Y.F. Song, L.X. Pei, L. Chen, et al., Electroacupuncture relieves irritable bowel syndrome by regulating IL-18 and gut microbial dysbiosis in a trinitrobenzene sulfonic acid-induced post-inflamatory animal model, Am. J. Chin. Med. 48 (2020) 77–90.
[15] Y. Ohe, SVKS, Risk factors of treatment-related death in chemotherapy and thoracic radiotherapy for lung cancer, Eur. J. Cancer 1 (2001) 54–63.
[16] L.B. Marks, XYZV, Radiation-induced lung injury, Semin. Radiat. Oncol. 3 (2003) 333–345.
[17] L. Meziani, M. Mondini, B. Petit, et al., CSF1R inhibition prevents radiation pulmonary fibrosis by depletion of interstitial macrophages, Eur. Respir. J. 51 (2018).
[18] C. Shih, S. Cheng, C. Wong, et al., Antiinflammatory and antihyperalgesic activity of C-phycocyanin, Anesth. Analg. 108 (2009) 1303–1310.
[19] J. Chen, K.S. Liu, T. Yang, et al., Spirulina and C-phycocyanin reduce cytotoxicity and inflammation-related expression of microglial cells, Nutr. Neurosci. 15 (2012) 252-256.

[20] K.F. Budden, S.L. Gellatly, D.L.A. Wood, et al., Emerging pathogenic links between microbiota and the gut-lung axis, Nat. Rev. Microbiol. 15 (2017) 55-63.

[21] R. Dzierski, S.Y. Park, D.R. Kashyap, et al., Pgyrp-regulated gut microflora pretovella faemini, parabacteroides distasonis and bacteroides eggerthi enhance and alstipes finegogdi attenuates colitis in mice, PLoS One 11 (2016), e146162.

[22] A. Lavelle, G. Lennon, O. O’Sullivan, et al., Spatial variation of the colonic microbiota in patients with ulcerative colitis and control volunteers, Gut 64 (2015) 1553-1561.

[23] A. Loy, C. Pfann, M. Steinberger, et al., Lifestyle and horizontal gene transfer-mediated evolution of mucispirillum schaedleri, a core member of the murine gut microbiota, Msystems (2017) 2.

[24] J. Koutouvaris, C. Zavos, S.A. Polyzos, et al., The gut-brain axis: interactions between Helicobacter pylori and enteric and central nervous systems, Annu. Gastroenterol. 28 (2015) 506.

[25] D.T. Smoot, How does helicobacter pylori cause mucosal damage? Direct mechanisms, Gastroenterology 113 (1997) S31–4.

[26] M.T. Whary, J.G. Fox, Detection, eradication, and research implications of Helicobacter infections in laboratory rodents, Lam. Anim. 35 (2006), 25-7, 30-6.

[27] C.L. Franklin, L.K. Riley, R.S. Livingston, et al., Enterohepatic lesions in SCID mice infected with Helicobacter bilis, Lab. Anim. Sci. 48 (1998) 334-339.

[28] D.M. Saultner, K. Riehel, T.A. Mistretta, et al., Gastrointestinal microbiome signatures of pediatric patients with irritable bowel syndrome, Gastroenterology 141 (2011) 1782-1791.

[29] Y. Kang, Y. Li, Y. Du, et al., Konjaku flour reduces obesity in mice by modulating the composition of the gut microbiota, Int. J. Obes. (Lond.) 43 (2019) 1631–1643.

[30] L.R. Lopesino, V. Petito, C. Graziani, et al., Gut microbiota in health, diverticular disease, irritable bowel syndrome, and inflammatory bowel diseases: time for microbial marker of gastrointestinal disorders, Digestive Dis. (Basel, Switzerland) 36 (2018) 56-65.

[31] Y. Liu, X. Wang, Q. Chen, et al., Camellia sinensis and lineea coreana ameliorate intestinal inflammation and modulate gut microbiota in dextran sulfate sodium-induced colitis mice, Mol. Nutr. Food Res. 64 (2020), e1900943.

[32] Y. Ge, S. Lin, B. Li, et al., Oxidized pork induces oxidative stress and inflammation by altering gut microbiota in mice, Mol. Nutr. Food Res. 64 (2020), e1901012.

[33] Z. Zhang, L. Lin, W. Liu, et al., Shen-Ling-Bai-Zhu-San alleviates functional dyspepsia in rats and modulates the composition of the gut microbiota, Nutr. Res. 71 (2019) 89-99.

[34] X. Chen, Q. Zuo, Y. Hai, et al., Lactulose: an indirect antioxidant ameliorating inflammatory bowel disease by increasing hydrogen production, Med. Hypotheses 76 (2011) 325–327.

[35] C.J. Meehan, R.G. Beiko, A phylogenomic view of ecological specialization in the Lachnospiraceae, a family of digestive tract-associated bacteria, Genome Biol. Evol. 6 (2014) 703-713.

[36] J. Li, B. Fang, G. Pang, et al., Age- and diet-specific effects of chronic exposure to chlorpyrifos on hormones, inflammation and gut microbiota in rats, Pestic Biochem. Phys. 159 (2019) 68-79.

[37] Y. Kang, D. Feng, H.K. Law, et al., Compositional alterations of gut microbiota in children with primary nephrotic syndrome after initial therapy, BMC Nephrol. 20 (2019) 434.

[38] F. Yan, N. Li, J. Shi, et al., Lactobacillus acidophilus alleviates type 2 diabetes by regulating hepatic glucose, lipid metabolism and gut microbiota in mice, Food Funct. 10 (2019) 5804-5815.

[39] M. Vital, A. Karch, D.H. Pieper, Colonic butyrate-producing communities in humans: an overview using omics data, Mycosystem 2 (2017) e117–30.

[40] A. Edwards, The mast cell and allergic diseases: role in pathogenesis and implications for therapy (vol 38, pg 1063, 2008), Clin. Exp. Allergy 38 (2012) 1242.

[41] K.V. Daughtry, S.D. Johanssonreier, R. Sanocky-Dawes, et al., Phenotypic and genotypic diversity of Lactobacillus buchneri strains isolated from spoiled, fermented cucumber, Int. J. Food Microbiol. 280 (2018) 46–56.

[42] R. Ashraf, N.P. Shah, Immune system stimulation by probiotic microorganisms, Crit. Rev. Food Sci. 54 (2014) 938–956.

[43] A.C. Ford, E.M. Quigley, B.E. Lacy, et al., Efficacy of prebiotics, probiotics, and synbiotics in irritable bowel syndrome and chronic idiopathic constipation: systematic review and meta-analysis, Am. J. Gastroenterol. 109 (2014) 1547–1561, 1546, 1562.

[44] R.T. Brink, M. Minekus, J.M.R.M. van der Vossen, et al., Antimicrobial activity of lactobacilli: preliminary characterization and optimization of production of acidocin B, a novel bacteriocin produced by Lactobacillus acidophilus M46, J. Bacteriol. 77 (1994) 140–148.

[45] K.X.T.D. Amruta Verma, B. Maria, Q.L. Grant, Expression of human ACE2 in Lactobacillus and beneficial effects in diabetic retinopathy in mice, Mol. Ther.–Nucleic Acids 5 (2019) 161–170.

[46] L. Si, R. Liu, Y. Jia, et al., Lactobacillus bulgaricus improves antioxidant capacity of black garlic in the prevention of gestational diabetes mellitus: a randomized control trial, Biotics. Rep. (2019) 39.

[47] J. Qin, Y. Li, Z. Cai, et al., A metagenome-wide association study of gut microbiota in type 2 diabetes, Nature 490 (2012) 55–60.

[48] N.M. Delzenne, P.D. Cani, A. Everard, et al., Gut microorganisms as promising targets for the management of type 2 diabetes, Diabetologia 58 (2015) 2206–2217.

[49] A. Moura-Assis, M.S. Afonso, V. de Oliveira, et al., Flaxseed oil rich in omega-3 protects aorta against inflammation and endoplasmic reticulum stress partially mediated by GPR120 receptor in obese, diabetic and dyslipidemic mice models, J. Nutr. Biochem. 53 (2018) 9–19.

[50] L. Zhu, L. Sha, K. Li, et al., Dietary flaxseed oil rich in omega-3 suppresses severity of type 2 diabetes mellitus via anti-inflammation and modulating gut microbiota in rats, Lipids Health Dis. 19 (2020) 16–20.

[51] J. Liu, S. Li, J. Liu, et al., Longitudinal characteristics of lymphocyte responses and cytokine profiles in the peripheral blood of SARS-CoV-2 infected patients, Ebiomedicine 55 (2020), 102763.

[52] N. Chen, M. Zhou, X. Dong, et al., Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study, Lancet 395 (2020) 507–513.

[53] L. Lin, X. Jiang, Z. Zhang, et al., Gastrointestinal symptoms of 95 cases with SARS-CoV-2 infection, Gut 69 (2020) 973–974.

[54] M.V. Parsey, R.M. Tudor, E. Abraham, Neutrophils are major contributors to intrapulmonary lung IL-1β expression after hemorrhage and endotoxemia, J. Immunol. 160 (1998) 1007–1013.