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Diseases & Research

An Analysis of Gut Bacterial and Fungal Community Interactions in *Saxifraga stolonifera* Curt.-Treated Mice

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Backgrounds: *Saxifraga stolonifera* Curt. is valued for its great medicinal potentials, especially for anti-inflammatory activity. The gut microbiome is gradually regarded as a pivotal part that contributes to the therapeutic efficacy of drugs and human health. Yet, research on the modulation of gut bacteria and gut fungi by *Saxifraga stolonifera* Curt. is rather scanty, which will hamper the understanding of the anti-inflammatory mechanism of *Saxifraga stolonifera* Curt.
Methods: To identify the regulatory effect of *Saxifraga stolonifera* Curt. on both gut bacteria and fungi, and gain insights into the interactions between gut bacteria and fungi, we treated C57BL/6 mice with aqueous extract of *Saxifraga stolonifera* Curt. (SSC group, 10 g/kg) and equivalent dose of distilled water (NC group), respectively. After 4 weeks treatment, fecal samples from each mouse were collected and then subjected to full-length 16S rRNA and ITS1/2 gene sequencing.
Results: As the results shown, *Saxifraga stolonifera* Curt. significantly enriched short chain fatty acid (SCFA)-producing bacteria, and reduced the abundance of pro-inflammatory bacteria such as *Alistipes*. Simultaneously, *Saxifraga stolonifera* Curt. increased the level of beneficial fungi such as *Mortierella kuhlmanii*, and decreased the abundance of pathogenic fungi such as *Fusarium sp.*. The correlation network presented a complex interaction between gut bacteria and fungi after *Saxifraga stolonifera* Curt. treatment.
Conclusions: Altogether, the current study, for the first time, displayed the regulatory effect of *Saxifraga stolonifera* Curt. on gut microbiome, which may be closely related to its pharmacological effects.
Keywords: Traditional Chinese medicine; *Saxifraga stolonifera* Curt.; Gut bacteria; Gut fungi; Microbial interaction.

Introduction

The dried herb of *Saxifraga stolonifera* Curt. (Hu-Er-Cao) is a widely used traditional medicines that contains a rich array of chemical component and exhibits potentials in dispelling pathogenic wind, clearing heat, and cooling blood to remove toxic substance[1]. Contemporary pharmacological investigations have revealed that *Saxifraga stolonifera* Curt. possesses a broad range of pharmacological activities, including anti-inflammatory, anti-bacterial, anti-tumor, cough-relieving, and hepatic protective effects[2-4]. Wang et al. have identified an active component isolated from *Saxifraga stolonifera* Curt. that can activate peroxisome proliferator activated receptor gamma (PPARγ), up-regulate silent information regulator factor 2-related enzyme 1 (SIRT1) expression, and inhibit acetylation of nuclear transcription factor kappB (NF-κB) p65, thereby attenuate experimental colitis in dextran sulfate sodium (DSS)-induced mice[5]. Furthermore, bergenin has the ability to modulate the production of Th1/Th2 cytokines and regulate the Toll-like receptor 2 (TLR 2)-mediated inflammatory response, thereby exerting anti-inflammatory activity and regulating the immune response[6,7]. Despite some knowledge regarding the pharmacological effects of *Saxifraga stolonifera* Curt. and its constituents, the underlying mechanisms remain incompletely elucidated. Therefore, current research should focus on updating related targets and precise mechanisms of *Saxifraga stolonifera* Curt. to enhance our understanding of its pharmacological effects and facilitate its clinical practice.

Gut microbiota represents a key mediator that plays crucial roles in maintaining human health, adjusting drugs' bioavailability and regulating signaling pathways. Emerging evidences have revealed that the gut microbiota is a necessary and sufficient factor for drug's efficacy, especially for TCMs. For example, Wu's research reported that the deletion of *Blautia* substantially abolished berebrine's hyperlipidemia-ameliorating efficacy[8]. This study further confirmed the indispensable role of the gut microbiota in drug's efficacy. In addition, our previous studies also revealed that gut microbiota was a vital bridge that mediated the efficacy of TCM formula[9] and its active component[10], and it can be served as a potential biological biomarker to reflect the property of TCMs[11-13]. Furthermore, a multicenter, open label RCT revealed that a TCM formula showed a better effect on gut microbiota in type 2 diabetes patients than metformin, which were res-

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ulted from the enrichment of *Faecalibacterium spp*.[14]. Therefore, the alteration of gut microbiota has gradually become a hotspot in TCM's efficacy and mechanism. Nevertheless, the modulation effects of *Saxifraga stolonifera* Curt. on gut microbiota still remain relatively unexplored, which will greatly limit the understanding for its mechanism.

Trillions of microorganisms, including bacteria, fungi, virus and archaea, inhabit the human gastrointestinal tract, forming a dynamic ecological community in the intestine, which is termed the "gut microbiome". With the development of sequencing technology, researchers have not limited themselves to investigate the role of gut bacteria, but began to pay attention to the contribution of gut fungi in human health. For instance, Lemoinne et al. found that the primary sclerosing cholangitis patients presented a dysbiosis of gut fungal community, characterized by an enhancement of *Exophiala* and a decline of *Saccharomyces cerevisiae*[15]. Moreover, Yu et al. revealed that the the ratio of Basidiomycota/Ascomycota was elevated in colorectal cancer patients, along with the increased abundance of Malasseziomycetes and decreased level of Saccharomycetes and Pneumocystidomycetes[16]. Simultaneously, they found 14 fungal biomarkers could distinguish colorectal cancer from healthy controls with an area under the receiver-operating characteristic curve (AUC) of 0.93[16], proving the crucial role of gut fungal community in human healthy. However, the current study on gut fungi still focused on the alteration of gut fungal composition during disease development, the modulation of symbiotic gut fungi by drugs, such as *Saxifraga stolonifera* Curt., still remains unclear.

It is not uncommon for bacteria and fungi to share a microhabitat in human intestine. As such, their interaction mode may be participated in the development of diseases and the mechanism of drugs. One study showed that the correlation between gut fungi and bacteria was mostly resulted from fungal *Ascomycota* and bacterial *Proteobacteria* in healthy controls, but were returned to mutually exclusive relationship in colorectal cancer patients[16], suggesting that the alteration of co-relationship between fungi and bacteria was an essential factor for developmengt of disease. Howbeit, a significant gap need to be filled to understand the co-occurrence interplay between gut bacteria and gut fungi after TCM treatment.

To address above issues, we investigated the modulation effect of several TCMs on gut bacterial and gut fungal community, along with explored the crosstalk between gut bacteria and fungi after TCM treatment. In current study, we mainly focused on the regulatory effect of *Saxifraga stolonifera* Curt. on gut microbiome. We fed mice with the aqueous extract of *Saxifraga stolonifera* Curt. for four weeks and detected their fecal bacteria and fungi using full-length 16S rRNA gene sequencing and ITS1/2 gene sequencing, respectively. This study not only revealed the regulatory effects of TCM on gut microbiome, but also provided a new direction for the elucidation of the pharmacological mechanism of TCM.

Methods

Preparation of *Saxifraga stolonifera* Curt. aqueous extract

Saxifraga stolonifera Curt. was purchased from Bohaotang Chinese traditional medicinal crops (Bozhou, China) and was authenticated by professor Nian Kai Zeng. The specimen was stored at Hainan Medical University with the number of FHMU6619. *Saxifraga stolonifera* Curt. aqueous extract was prepared according to previous method. Firstly, the total of 200 g raw medicinal materials was subjected to 1000 mL distilled water to decoct for half an hour. Then the solution was remained and the residue was subjected to another 1000 mL distilled water to decoct for half an hour again. After extraction, merged the extracts and filtered, concentrated to obtain the extract of *Saxifraga stolonifera* Curt. (200 mL).

Animals and treatment

All of the experimental procedures in this study were followed the guidelines for the care and use of laboratory animals of the National Institutes of Health, and were fully approved by the Medical Ethics Committee of Hainan Medical University (No. SYXK-2017-0013).

Sixteen male C57BL/6 mice (20–22 g, 6–8 weeks) were purchased from GemPharmatech Co. LTD (Haikou, China). The mice were housed in an SPF environment with a controlled conditions (humidity: 40 ± 5%; temperature: 23 ± 2 ℃; 12 h light/dark cycle). After adaptive feeding for one week, the animals were randomly and evenly divided into two groups, including control group (NC, N = 8) and *Saxifraga stolonifera* Curt. group (SSC, N = 8). The mice in NC group and SSC group were orally administrated with distilled water (10 g/kg) and SSC aqueous extract (10 g/kg) respectively. The dosage of SSC was converted equivalently according to its recommended clinical dosage in Pharmacopoeia of the People's Republic of China (2020 edition). After four weeks treatment, each mouse was placed in a clean cage and allowed to defecate spontaneously. The fresh feces was immediately collected in a freezing tube and froze in liquid nitrogen, and then transferred to −80 ℃ for further gut bacteria and gut fungi detection.

DNA extraction and gene sequencing

The bacterial or fungal DNA of each mouse was extracted from its fecal sample using FastDNA Spin Kit (MP Biomedicals, Santa Ana, CA, USA) according to the manufacturer's instruction. The V1–V9 region of 16S rRNA gene and ITS1/2 region of fungal gene were amplified by TransGen AP221-02 and TransStart Fastpfu DNA Polymerase. The PacBio libraries were built, and the bacterial and fungal gene sequence was sequenced by an Illumina HiSeq 2500 instrument according to the manufacturer's instructions.

Bioinformatics analysis

The full-length 16S rRNA and ITS1/2 sequencing data were subjected to the quality control, assembly and abundance quantification. Alpha-diversity and beta-diversity of gut microbiome were analyzed by vegan package (v2.7) of R version 4.0.2.. The difference of Bray-Curtis distance of principal coordinates analysis (PCoA) and clustering analysis at OTU level was assessed by Adonis analysis. Correlation analysis was calculated by spearman algorithm. The differences among groups were analyzed by Student's t-test and $P < 0.05$ was considered as statistical significance.

Results

Saxifraga stolonifera Curt. showed minor influence on gut bacterial diversity and structure

To investigate the influence of *Saxifraga stolonifera* Curt. on gut bacterial diversity and structure, we first assessed the difference of Chao1, ACE, Shannon and Simpson indices between NC group and *Saxifraga stolonifera* Curt. (SSC) group. As shown in Fig. 1, the above four indices had no significant difference among two groups (Fig. 1 A–D). We also performed principal coordinate analysis (PCoA) and hierarchical cluster analysis to detect the structural change of gut bacteria after *Saxifraga stolonifera* Curt. administration. The diagram showed that the structure of gut bacteria in SSC group was just slightly separated from NC group (Fig. 1 E, F). These results revealed that *Saxifraga stolonifera* Curt. showed minor influence on gut bacterial diversity and structure.

Saxifraga stolonifera Curt. altered gut bacterial composition

We then detected the gut bacterial composition at the phylum, genus and species levels to identify the difference of gut bacteria among two groups. The composition profile at the phylum level showed that the dominant bacteria were Firmicutes, Bacteroidetes and Verrucomicrobia (Fig. 2A). The relative abundance of Firmicutes was increased but Bacteroidetes was decreased after *Saxifraga stolonifera* Curt. treatment, suggesting that *Saxifraga stolonifera* Curt. could increase Firmicutes/Bacteroidetes (F/B) ratio. At the genus level, *Saxifraga stolonifera* Curt. enhanced the level of *Lactobacillus*, *Kineothrix* and *Schaedlerella*, whereas reduced *Muribaculum*, *Mucispirillum*, *Blautia* and *Alistipes* (Fig. 2B). At the species level, *Lactobacillus johnsonii*, *Kineothrix alysoides*, *Lactobacillus gasseri* and *Lactobacillus paragasseri* were enriched by *Saxifraga stolonifera* Curt., while *Muribaculum intestinale* and *Mucispirillum schaedleri* were decreased (Fig. 2C).

The intergroup difference analysis further displayed the differential gut bacterial species regulated by *Saxifraga stolonifera* Curt.. As compared to NC group, fifteen species, including *Lachnotalea sp900184995*, *Erysipelatoclostridium cocleatum*, *Adlercreutzia equolifaciens*, *Anaerostipes sp001940315*, *Faecalibacillus sp003480255*, *Roseburia sp900552665*, *Monoglobus_uncultured bacterium*, *Intestinimonas timonensis*, *Dubosiella sp000403415*, *Massilioclostridium coli*, *Roseburia_uncultured bacterium*, *Ruminococcus_uncultured bacterium*, *Petroclostridium xylanilyticum*, *Lawsonibacter sp000177015* and *Lactobacillus gasseri*, were up-regulated in SSC group, while eleven gut bacterial species, including *Alistipes timonensis*, *Desulfovibrio sp. Marseille-P3199*, *Alistipes dispar*, *Alistipes sp900290115*, *Muribaculum intestinale*, *Tidjanibacter inops_A*, *Alistipes putredinis*, *Ligilactobacillus animalis*, *Rikenella microfusus*, *Pseudoclostridium thermosuccinogenes* and *Duncaniella freteri*, were down-regulated in SSC group (Fig. 2D). The linear discriminant analysis effect size (LEfSe) analysis identified the characteristic bacteria between SSC and NC group. The results showed

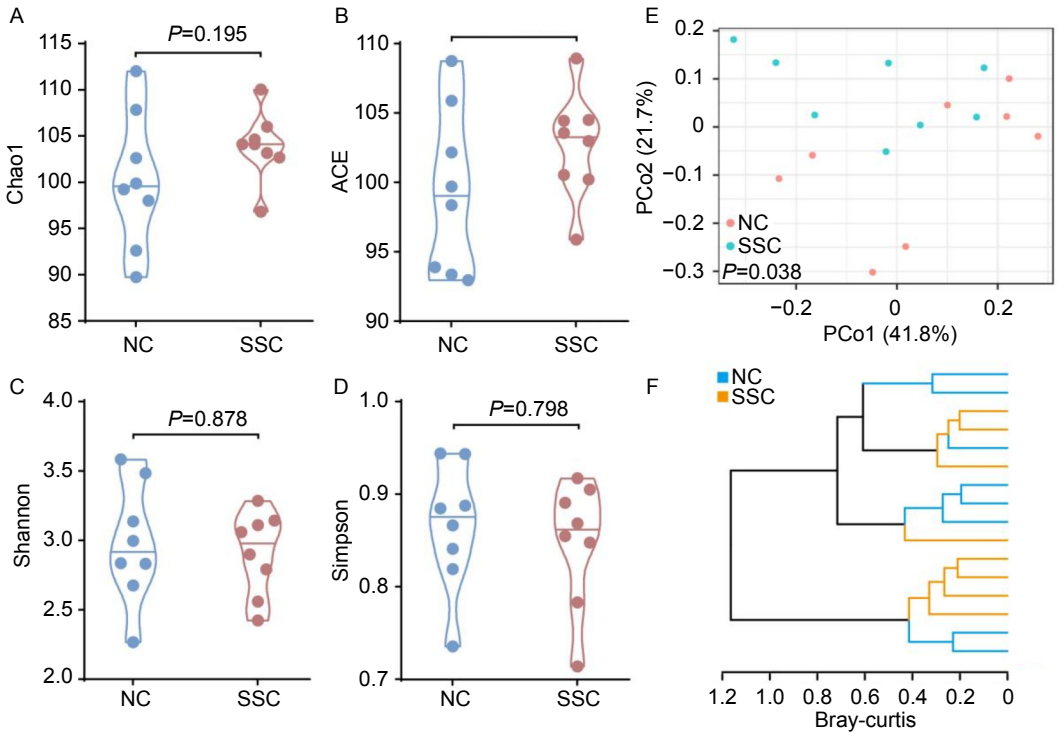

Fig. 1. Effect of *Saxifraga stolonifera* Curt. on gut bacterial diversity.
A. The alpha-diversity of gut bacteria was assessed by Chao1 index.
B. The alpha-diversity of gut bacteria was assessed by ACE index.
C. The alpha-diversity of gut bacteria was assessed by Shannon index.
D. The alpha-diversity of gut bacteria was assessed by Simpson index.
E. The structure of gut bacteria was assessed by principal coordinate analysis (PCoA).
F. Hierarchical cluster analysis.

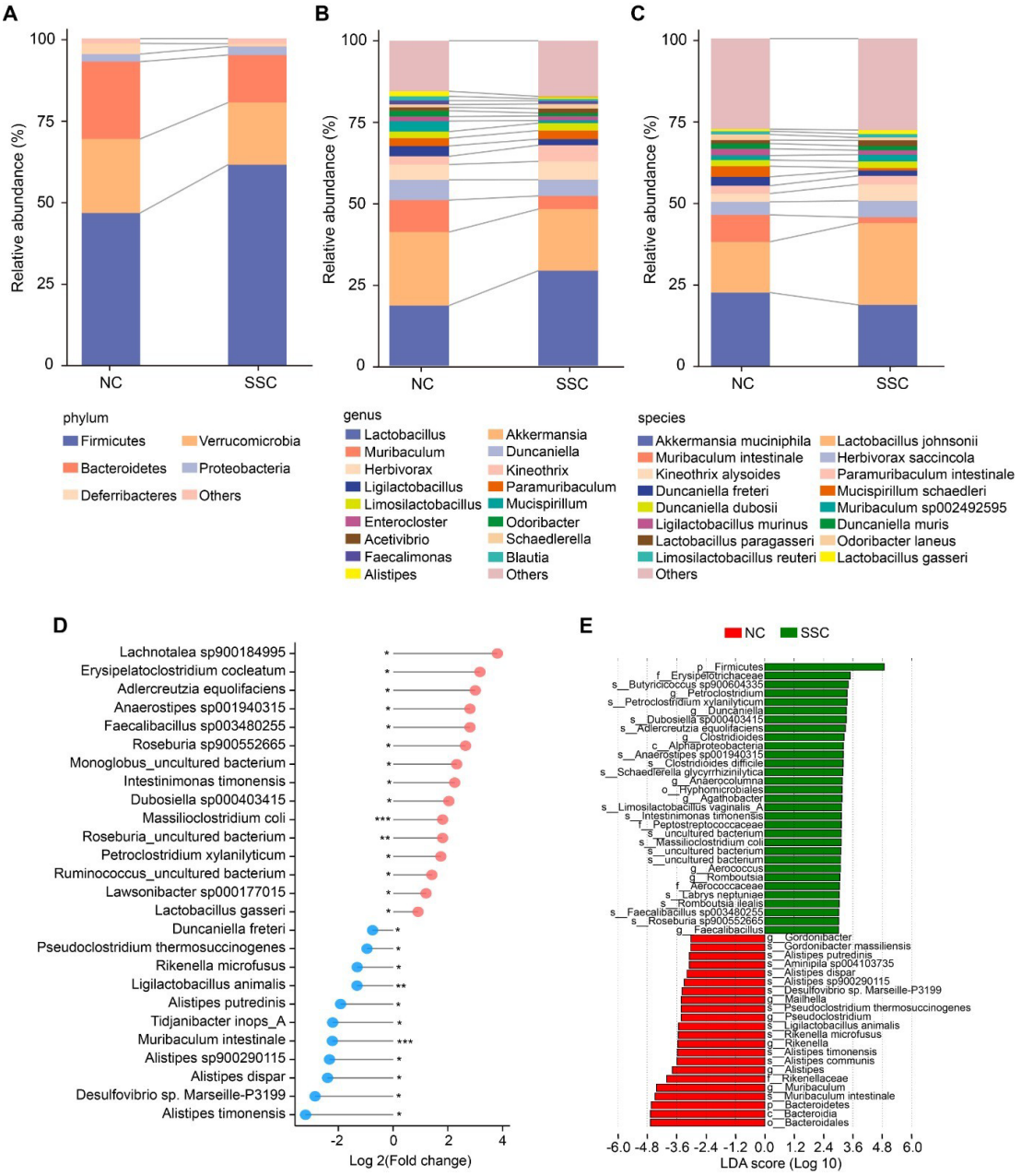

Fig. 2. Effect of *Saxifraga stolonifera* Curt. on gut bacterial composition.
A. The gut bacterial composition at the phylum level.
B. The gut bacterial composition at the genus level.
C. The gut bacterial composition at the species level.
D. The differential gut bacterial species identified by Student's t-test.
E. Distinct bacteria identified in SSC and NC group via LEfSe analysis.
*P < 0.05, **P < 0.01, ***P < 0.001.

that *Butyricicoccus sp900604335*, *Petroclostridium xylanilyticum*, *Dubosiella sp000403415*, *Adlercreutzia equolifaciens*, *Anaerostipes sp001940315*, *Schaedlerella glycyrrhizinilytica*, *Limosilactobacillus vaginalis_A*, *Intestinimonas timonensis*, *Massilioclostridium coli*, *Labrys neptuniae*, *Romboutsia ilealis*, *Faecalibacillus sp003480255* and *Roseburia sp900552665* were enriched by *Saxifraga stolonifera* Curt., whereas *Gordonibacter massiliensis*, *Alistipes putredinis*, *Aminipila sp004103735*, *Alistipes dispar*, *Alistipes sp900290115*, *Desulfovibrio sp. Marseille-P3199*, *Pseudoclostridium thermosuccinogenes*, *Ligilactobacillus animalis*, *Rikenella microfusus*, *Alistipes timonensis*, *Alistipes communis* and *Muribaculum intestinale* were more enriched in NC group (Fig. 2E).

Saxifraga stolonifera Curt. decreased the diversity of gut fungal community

Recent researches have highlighted a fact that gut fungi conveys

beneficial effects to symbiotic host via enhancing thesystemic immunity, and preventing the invasive infections[17]. Consequently, we also monitored the alteration of gut fungal after *Saxifraga stolonifera* Curt. treatment using ITS1/2 gene sequencing. Based on the data of Chao1, ACE, Shannon and Simpson indices, it could be seen that *Saxifraga stolonifera* Curt. significantly decreased ACE, but exerted little effect on Chao1, Shannon and Simpson (Fig. 3A–D), implying that *Saxifraga stolonifera* Curt. treatment only weakly reduced the alpha-diversity of gut fungi. PCoA and hierarchical cluster analysis based on bray-curtis distance displayed that the SSC group was markedly separated from NC group (Fig. 3E, F). The above results implied that *Saxifraga stolonifera* Curt. could slightly decrease the diversity of gut fungi and significantly changed its structure.

Saxifraga stolonifera Curt. altered gut fungal composition

Then, the gut fungal composition among SSC and NC group was detected at the phylum, genus and species level, respectively. We found Ascomycota, Basidiomycota and Mucoromycota were the dominant fungal phyla in two groups, where Ascomycota was decreased while Basidiomycota was increased in the SSC group (Fig. 4A). In profile diagram at the genus levels, we found *Purpureocillium* and *Cephaliophora* were reduced, but *Neosartorya*, *Cladosporium* and *Alternaria* were enhanced by *Saxifraga stolonifera* Curt.. (Fig. 4B). At the species level, *Saxifraga stolonifera* Curt. decreased the abundance of *Purpureocillium lilacinum*, *Aspergillus penicillioides*, *Cephaliophora tropica*, *Mortierella sp. MEL 2385001*, *Fusarium sp.* and *Penicillium janthinellum*, but increased the abundance of *Neosartorya sp*., *Cladosporium sp*., *Alternaria sp*. and *Aspergillus ruber* (Fig. 4C). The further intergroup difference analysis unveiled that eight fungi, *Aspergillus oryzae*, *Aspergillus chevalieri*, *Penicillium sp. BAB-5649*, *Penicillium janthinellum*, *Penicillium sp. GZU-BCECYN66-5*, *Fusarium sp.*, *Mortierella alpina* and *Minimedusa polyspora*, were reduced by *Saxifraga stolonifera* Curt. (Fig. 4D), but two fungi, *Alternaria sp.* and *Mortierella kuhlmanii*, were enhanced by *Saxifraga stolonifera* Curt. (Fig. 4E).

The interplay of gut fungi and gut bacteria community in *Saxifraga stolonifera* Curt.-treated mice

Gut bacteria and fungi jointly inhabit the intestine so that they are inevitably in a symbiotic or competitive relationship. To further investigate the correlation of gut bacteria and gut fungi in *Saxifraga stolonifera* Curt.-treated mice, we performed association analysis between bacteria and fungi based on Spearman algorithm. The interaction network depicted the complex interactions between gut bacteria and gut fungi. Overall, bacteria of *Muribaculum intestinale*, *Ligilactobacillus animalis* and *Alistipes putredinis* were positively correlated with fungi of *Purpureocillium lilacinum*, *Candida sp. (in: Saccharomycetales)*, *Chaetomium globosum*, *Trichoderma harzianum* and *Eupenidiella venezuelensis*. In contrary, bacteria of *Massilioclostridium coli*

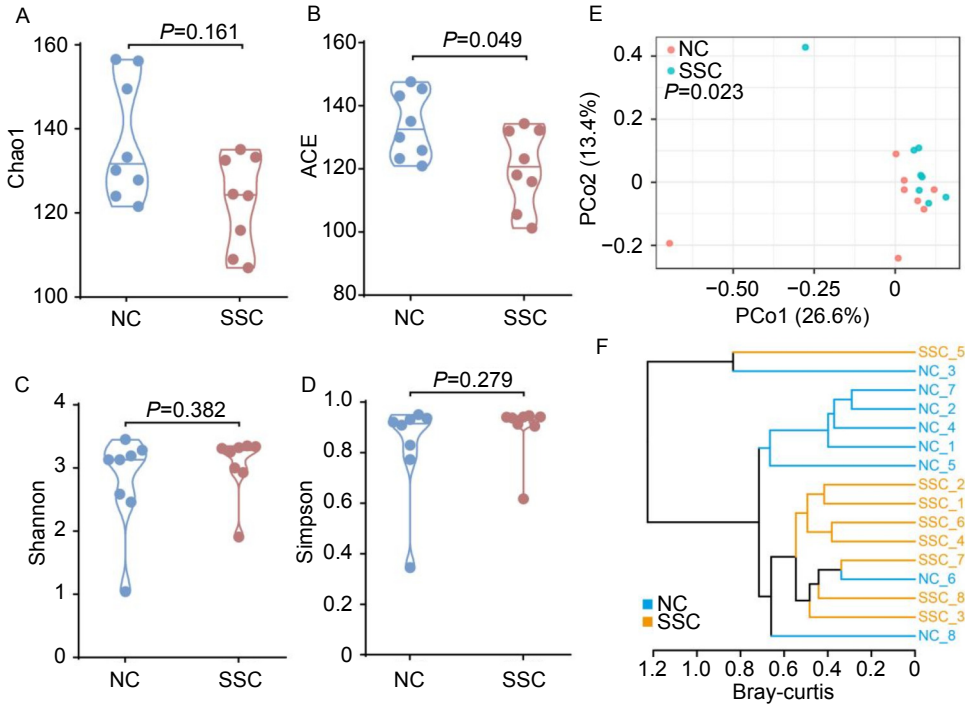

Fig. 3. Effect of *Saxifraga stolonifera* Curt. on gut fungal diversity.
A. The alpha-diversity of gut fungi was assessed by Chao1 index.
B. The alpha-diversity of gut fungi was assessed by ACE index.
C. The alpha-diversity of gut fungi was assessed by Shannon index.
D. The alpha-diversity of gut fungi was assessed by Simpson index.
E. The structure of gut fungi was assessed by principal coordinate analysis (PCoA).
F. Hierarchical cluster analysis.

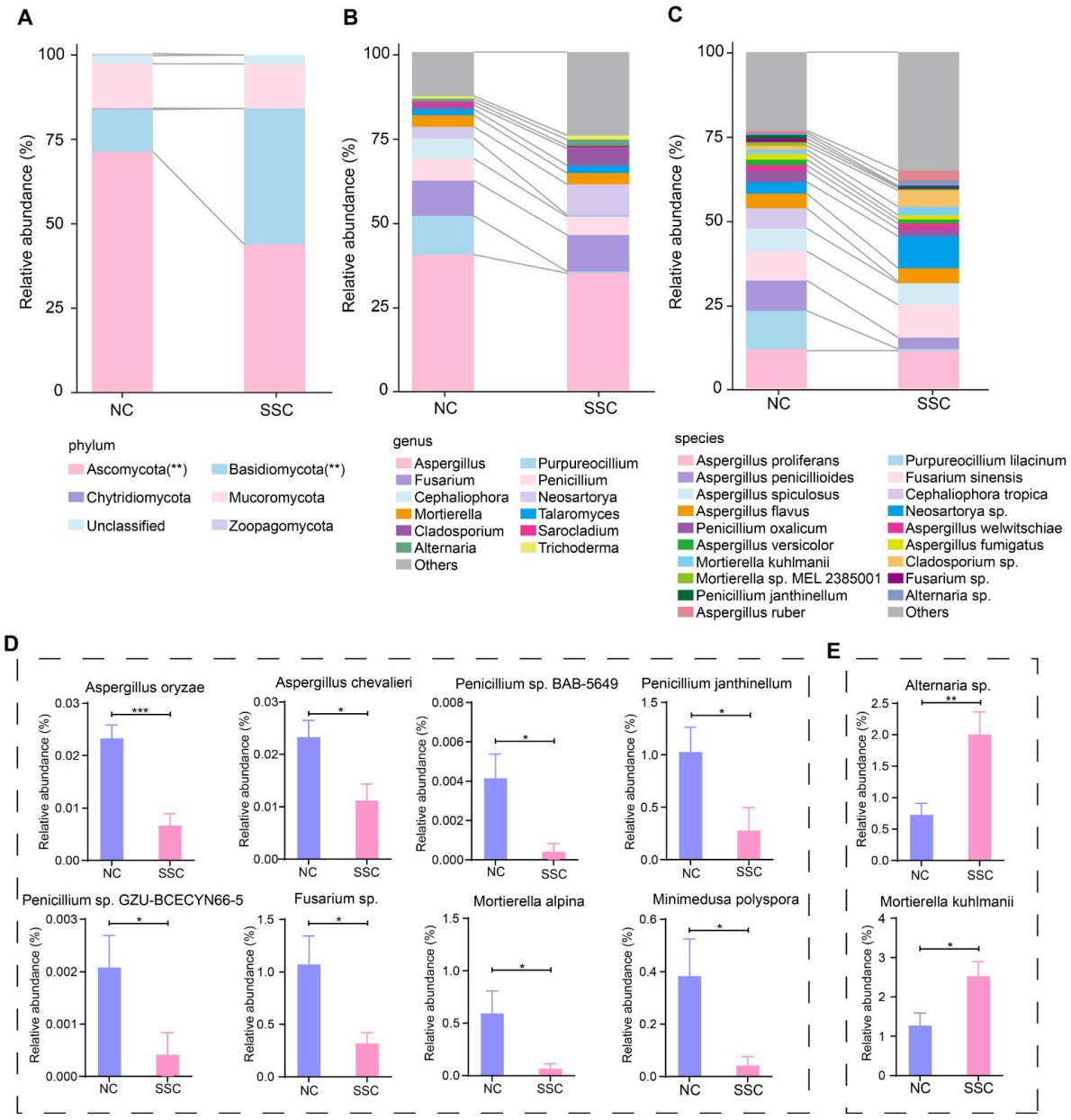

Fig. 4. Effect of *Saxifraga stolonifera* Curt. on gut fungal composition.
A. The gut fungal composition at the phylum level.
B. The gut fungal composition at the genus level.
C. The gut fungal composition at the species level.
D. The down-regulated fungal species by *Saxifraga stolonifera* Curt..
E. The up-regulated fungal species by *Saxifraga stolonifera* Curt..
*P < 0.05, **P < 0.01, ***P < 0.001.

was negatively related to *Nigrograna sp. FL-2018a*, *Trichoderma asperellum* and *Solicoccozyma aeria*. Similarly, gut fungi of *Penicillium citreonigrum* was also negatively associated with bacteria of *Agathobacter rectalis*, *Prevotellamassilia sp0029-33955* and *Lacrimispora sp000526575*. Additionally, gut bacterial species *Duncaniella sp001689575*, Lachnospiraceae_*Unclassified*, *Roseburia sp001940165*, *Dysosmobacter welbionis* and *Roseburia_uncultured bacterium* showed negative association with fungal species of *Sarocladium spinificis*, *Cataractispora appendiculata*, *Penicillium polonicum*, *Cladosporium cladosporioides* and *Gemmina gemmarum,* respectively. Simultaneously, bacteria of *Desulfovibrio sp011039135*, *Enterocloster sp001517625*, *Gordonibacter massiliensis*, *Herbinix sp01417-4405*, *Mesorhizobium terrae*, *Limosilactobacillus_uncultured bacterium*, *Rikenella microfusus* and *Alistipes sp900290115* were positively associated with fungi of *Occultifur sp. TMS-2011*, *Abundisporus mollissimus*, *Sagenomella oligospora*, *Paraconiothyrium fuckelii*, *Penicillium sp.*, *Hypoxylon monticulosum*, *Tetracladium psychrophilum* and *Fragosphaeria purpurea*, respectively (Fig. 5).

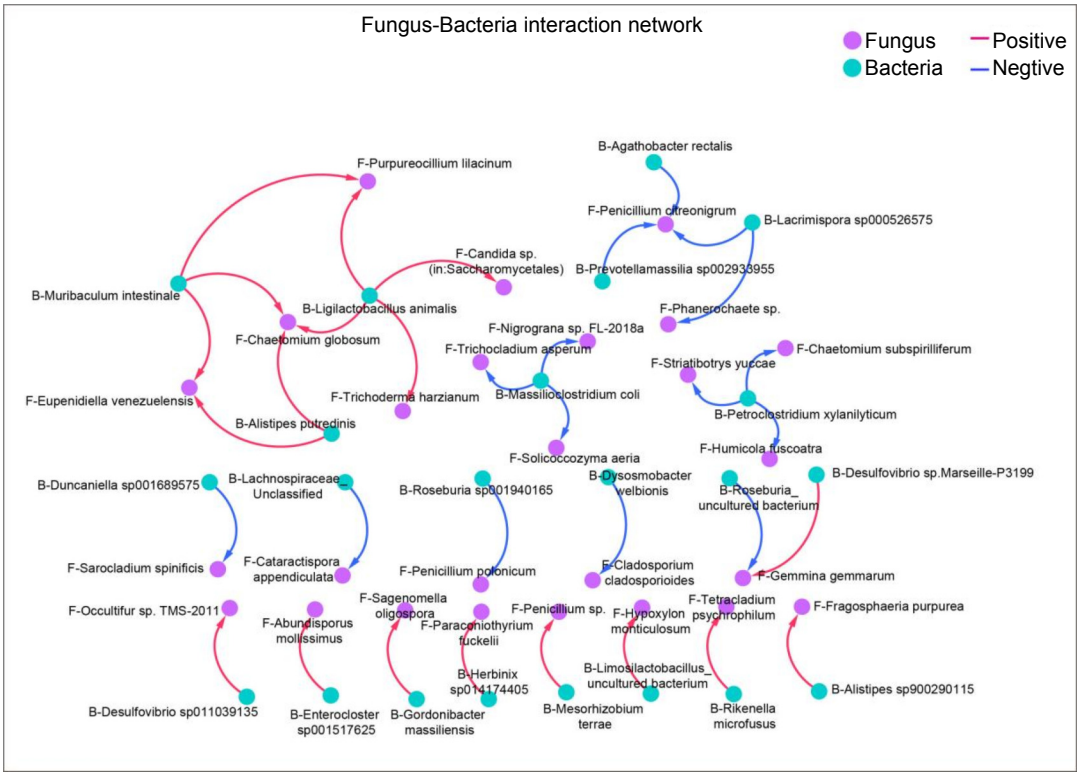

Fig. 5. The correlation network between gut bacteria and gut fungi.
Red line represents positive correlation, while blue line represents negative correlation. *P < 0.05, **P < 0.01.

Discussion

Gut microbiome is gradually being regarded as one of the potential signals for drug's mechanism and disease course. In this study, we investigated the modulation of *Saxifraga stolonifera* Curt. on both gut bacterial and gut fungal community, along with reconnoitred the mutual interplay of gut fungi and bacteria in *Saxifraga stolonifera* Curt.-conditioned mice. The results showed that *Saxifraga stolonifera* Curt. changed gut fungal structure but exerted minor effect on gut bacterial diversity and structure. *Saxifraga stolonifera Curt.* not only up-regulated SCFA-producing bacteria, but also down-regulated *Alistipes (A. timonensis*, *A.dispar*, *A. sp900290115*, *A. putredinis*), *Aspergillus* (*A. oryzae*, *A. chevalieri*) and *Penicillium* (*P. sp. BAB-5649*, *P. janthinellum*, *P. sp. GZU-BCECYN66-5*). Additionally, we found the complex interaction was presented in *Saxifraga stolonifera* Curt.-conditioned mice. To the best of our knowledge, this is the first time to demonstrate the modulation of gut bacteria and fungi by *Saxifraga stolonifera* Curt.

Gut microbiota influence host health mainly through their active metabolites such as short chain fatty acid (SCFA), bile acids (BAs), indole and its derivatives, and so on[18-20]. SCFAs are the end products of dietary fiber metabolized by gut microbiota, and are proved to be participate in host inflammation[21]. Hence, the production of SCFAs is linked with immune function and excessive immune responses, further mediates the pharmacological efficacy of TCMs[22,23]. In present study, *Saxifraga stolonifera* Curt. enriched SCFA-producing bacteria, such as *Anaerostipes*, *Roseburia*, *Dubosiella*, *Roseburia*, *Ruminococcus* and *Lactobacillus gasseri*, suggesting that *Saxifraga stolonifera* Curt. may exert anti-inflammatory and immunomodulatory effect through promoting the secretion of SCFA[20], but this needs to be further verified through measuring the content of SCFAs. Additionally, we found *Saxifraga stolonifera* Curt. decreased the abundance of *Alistipes* (*A. timonensis*, *A. dispar*, *A. sp900290115*, *A. putredinis*). As *Alistipes* is mostly isolated from patients with appendicitis, abdominal and rectal abscesses[24], the species of *Alistipes* are mostly associated with the inflammatory reaction, revealing that the reduction of *Alistipes* by *Saxifraga stolonifera* Curt. is also related to its anti-inflammatory activity. Nevertheless, these results were obtained from normal mice and the next step should put forward to explore how *Saxifraga stolonifera* Curt. influence the composition of gut bacteria in pathological models.

At present, the studies on gut fungi are still in the initial stage, so it is not clear about the physiological significance of gut fungi changed by TCM. In this study, gut fungi mainly enriched in mice after *Saxifraga stolonifera* Curt. treatment were Ascomycota and Basidiomycota, which is consistent with the composition of fungi in human body[25]. Meanwhile, we found *Saxifraga stolonifera* Curt. decreased the relative abundance of *Aspergillus oryzae*, *Aspergillus chevalieri*, *Penicillium sp. BAB-5649*, *Penicillium janthinellum*, *Penicillium sp. GZU-BCECYN66-5*, *Fusarium sp.*, *Mortierella alpina* and *Minimedusa polyspora*, while increased *Alternaria sp.* and *Mortierella kuhlmanii*. *Mortierella kuhlmanii* is usually considered as a beneficial fungus, while *Fusarium sp.* is a pathogenic fungus[26], thus we speculated that this alteration of gut fungi by *Saxifraga stolonifera* Curt. may involve in its pharmacological effect. Due to most studies to date

have overlooked the function of gut fungi, the physiological significance caused by specific fungal species remains largely unknown. Therefore, future studies should compensate for these deficiencies to promote the understanding of drug's efficacy and related mechanism.

For the first time in current study, we monitored the interactions between gut bacteria and fungi in *Saxifraga stolonifera* Curt.-conditioned mice. The correlation analysis depicted the complex interplay between gut fungi and bacteria. *Penicillium citreonigrum* possessed fructofuranosidase that could produce fructooligosaccharides to exert antibacterial potential[27]. In present study, we found *Penicillium citreonigrum* was negatively associated with bacteria of *Agathobacter rectalis*, *Prevotellamassilia sp002933955* and *Lacrimispora sp000526575*, implying that the alteration of *Penicillium citreonigrum* by *Saxifraga stolonifera* Curt. may influence the growth of gut bacteria. Due to the less knowledge about the physiological function of gut fungi, along with the potential implication about the interaction between gut fungi and bacteria, future studies should focus and address these issues timely.

We certainly admitted some limitations presented in the present study, that is, the normal mice are employed in the experiment, which cannot directly prove the relationship between the alteration of gut bacteria and the efficacy of *Saxifraga stolonifera* Curt.. Howbeit, we described the modulation of *Saxifraga stolonifera* Curt. on gut bacterial and gut fungal community for the first time, which provided the preliminary understanding of the alteration of gut bacteria and gut fungi after *Saxifraga stolonifera* Curt. treatment. Furthermore, we proposed, at least, one hypothesis that the interactions between gut fungi and bacteria may be linked with the pharmacological effect of *Saxifraga stolonifera* Curt.'s. The further research on the underlying mechanism of TCM should take gut microbiome as one of the factors to considerate.

Conclusion

Altogether, the results of this study suggested that the enrichment of SCFA producers and the reduction of pro-inflammatory bacteria by *Saxifraga stolonifera* Curt. may be involved in its anti-inflammatory mechanism. This study complemented previous studies that highlight the impact of drugs on gut microbiome. Further, this research provided a mechanistic study direction for *Saxifraga stolonifera* Curt. to some extent.

Abbreviations

DSS, dextran sulfate sodium; NF-κB, nuclear transcription factor kappB; PCoA, principal coordinate analysis; PPARγ, peroxisome proliferator activated receptor gamma; SCFA, short chain fatty acid; SIRT1, silent information regulator factor 2-related enzyme 1; TCM, traditional Chinese medicine; TLR2, toll-like receptor 2.

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Authors' contributions

YNY analyzed data, prepared figures and wrote the manuscript. XHZ and LEZ edited the manuscript. WYL and XPZ were responsible for the concept and design of the study. CMW designed the experiments, wrote and edited the manuscript.

References

[1] Xie ZP. A Dictionary of Traditional Chinese Medicine: First Volume. Shanghai Scientific and Technical Publishers: Shanghai, China, 1977.

[2] He HM, Dai Y, Xia YF. Research progress of herba Saxifragae. Chin Wild Plant Res, 2017, 36(02): 75-78.

[3] Liu D, Yang P, Zhang YQ. Water-soluble extract of Saxifraga stolonifera has anti-tumor effects on Lewis lung carcinoma-bearing mice. Bioorg Med Chem Lett, 2016, 26(19): 4671-4678.

[4] Zhang H, Li QY, He SW, et al. Advance in chemical constituents and pharmacological activities of Saxifraga stolonifera. Prog Veterinary Med, 2021, 42(01): 94-99.

[5] Wang K, Li YF, Lv Q, et al. Bergenin, acting as an agonist of PPARgamma, ameliorates experimental colitis in mice through improving expression of SIRT1, and therefore inhibiting NF-kappaB-mediated macrophage activation. Front Pharmacol, 2017, 8: 981.

[6] Nazir N, Koul S, Qurishi MA, et al. Immunomodulatory effect of bergenin and norbergenin against adjuvant-induced arthritis-a flow cytometric study. J Ethnopharmacol, 2007, 112(2): 401-405.

[7] Kawahara T, Ito A, Kiso A, et al. Inhibitory effect of strawberry geranium (Saxifraga stolonifera) on Toll-like receptor 2-mediated inflammatory response in human skin keratinocytes. J Ethnopharmacol, 2021, 275: 114039.

[8] Wu CM, Zhao Y, Zhang YY, et al. Gut microbiota specifically mediates the anti-hypercholesterolemic effect of berberine (BBR) and facilitates to predict BBR's cholesterol-decreasing efficacy in patients. J Adv Res, 2022, 37: 197-208.

[9] Wang XH, Yang YN, Liang Y, et al. Structural modulation of gut microbiota during alleviation of experimental passive Heymann nephritis in rats by a traditional Chinese herbal formula. Biomed Pharmacother, 2022, 145: 112475.

[10] Li YG, Xu WY, Zhang F, et al. The gut microbiota-produced indole-3-propionic acid confers the antihyperlipidemic effect of mulberry-derived 1-deoxynojirimycin. mSystems, 2020, 5(5): e00313-00320.

[11] Zhang XP, Yang YN, Zhang F, et al. Traditional Chinese medicines differentially modulate the gut microbiota based on their nature (Yao-Xing). Phytomedicine, 2021, 85: 153496.

[12] Yang YN, Deng YT, Zang CC, et al. The Gut Microbial Co-Abundance Gene Groups (CAGs) Differentially Respond to the Flavor (Yao-Wei) of Chinese Materia Medica. Am J Chin Med, 2022, 50(8): 2223-2244.

[13] Yang Y, Su W, Zang C, et al. Traditional Chinese medicines (TCMs) with varied meridians (Gui-Jing) differentially alleviate the adverse impact of Coptis chinensis on gut microbiota. J Ethnopharmacol, 2023, 307: 116256.

[14] Tong X, Xu J, Lian F, et al. Structural Alteration of Gut Microbiota during the Amelioration of Human Type 2 Diabetes with Hyperlipidemia by Metformin and a Traditional Chinese Herbal Formula: a Multicenter, Randomized, Open Label Clinical Trial. mBio, 2018, 9(3): e02392-02317.

[15] Lemoinne S, Kemgang A, Ben Belkacem K, et al. Fungi participate

in the dysbiosis of gut microbiota in patients with primary sclerosing cholangitis. Gut, 2020, 69(1): 92-102.

[16] Coker OO, Nakatsu G, Dai RZ, et al. Enteric fungal microbiota dysbiosis and ecological alterations in colorectal cancer. Gut, 2019, 68(4): 654-662.

[17] Shao TY, Haslam DB, Bennett RJ, et al. Friendly fungi: symbiosis with commensal Candida albicans. Trends Immunol, 2022, 43(9): 706-717.

[18] Zhan K, Zheng H, Li JQ, et al. Gut microbiota-bile acid crosstalk in diarrhea-irritable bowel syndrome. Biomed Res Int, 2020, 2020: 3828249.

[19] Li YY, Zhang YM, Wei KX, et al. Review: Effect of gut microbiota and its metabolite SCFAs on radiation-induced intestinal injury. Front Cell Infect Microbiol, 2021, 11: 577236.

[20] Agus A, Planchais J, Sokol H. Gut microbiota regulation of tryptophan metabolism in health and disease. Cell Host Microbe, 2018, 23(6): 716-724.

[21] Xu L, Li W, Chen SY, et al. Oenothein B ameliorates hepatic injury in alcoholic liver disease mice by improving oxidative stress and inflammation and modulating the gut microbiota. Front Nutr, 2022, 9: 1053718.

[22] Zhang ZL, Zhang H, Chen T, et al. Regulatory role of short-chain fatty acids in inflammatory bowel disease. Cell Commun Signal, 2022, 20(1): 64.

[23] Zhang F, Zhang XP, Yu JQ, et al. The gut microbiota confers the lipid-lowering effect of bitter melon (Momordica charantia L.) In high-fat diet (HFD)-Induced hyperlipidemic mice. Biomed Pharmacother, 2020, 131: 110667.

[24] Parker BJ, Wearsch PA, Veloo ACM, et al. The genus Alistipes: gut bacteria with emerging implications to inflammation, cancer, and mental health. Front Immunol, 2020, 11: 906.

[25] Huseyin CE, O'Toole PW, Cotter PD, et al. Forgotten fungi-the gut mycobiome in human health and disease. FEMS Microbiol Rev, 2017, 41(4): 479-511.

[26] Sun J, Luo HM, Yu Q, et al. Optimal NPK fertilizer combination increases Panax ginseng yield and quality and affects diversity and structure of rhizosphere fungal communities. Front Microbiol, 2022, 13: 919434.

[27] Araújo VPB, Araújo TK, Sousa KMN, et al. A novel β-fructofuranosidase produced by Penicillium citreonigrum URM 4459: purification and biochemical features. Prep Biochem Biotechnol, 2022: 1-8.