

# Identification and functional analysis of bacteria in sclerotia of *Cordyceps militaris*

**Li Luo**

Guizhou University

**Jia-Xi Zhou**

Guizhou University

**Zhong-Shun Xu**

Guizhou University

**Ying-Ming Gao**

Guizhou University

**Xiao Zou** (✉ [xzou@gzu.edu.cn](mailto:xzou@gzu.edu.cn))

Guizhou University

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## Research Article

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# Abstract

## Background

*Cordyceps militaris* is a fungus that parasitizes insects. Compounds from *C. militaris* are valuable in medicine and functional food. There are many kinds of bacteria in the natural sclerotia of *C. militaris*. However, the community structure of microorganisms in samples from different places may be different, and the corresponding ecological functions require experimental verification.

## Results

We used high-throughput sequencing technology to analyze bacterial 16S rRNA gene sequences in sclerotia of three samples of *C. militaris* from Liaoning Province, China. *Pseudomonas*, *Pedobacter*, *Sphingobacterium*, and *Serratia* were the dominant bacterial genera in the sclerotia. *Pseudomonas* and *Pedobacter* are heterotrophic, *Sphingobacterium* decomposes urea, and *Serratia* reduce nitrate. Two strains of bacteria, N-2 and N-26, were isolated from the sclerotia of the *C. militaris*, cultured, biochemically characterized, and identified as *Stenotrophomonas maltophilia* and *Pseudomonas baetica*, respectively. When these isolated strains were respectively cocultured with *C. militaris*, the mycelium biomass, mycelium pellet diameter, and cordycepin content decreased.

## Conclusions

Bacteria in sclerotia have an important effect on the growth of *C. militaris* and the production of its metabolites.

## Background

*Cordyceps militaris* is a member of the fungal genus *Cordyceps* and is a traditional Chinese medicine, containing a variety of active substances including cordycepin (3'-deoxyadenosine), cordyceps polysaccharide, and cordyceps acid [1]. In humans, cordycepin and cordyceps polysaccharide improve immunity; protect the kidney; have antifatigue and antioxidation properties; inhibit bacterial growth, inflammation, and tumors; and can be used as an effective anticancer supplement [2]. Cordycepin has the potential to be used against COVID-19 [3]. Much attention has been paid to the development of *C. militaris* [4, 5].

*C. militaris* parasitizes insect larvae [6]. It is distributed throughout the Northern Hemisphere [7]. Its host insects include Lepidoptera, Coleoptera, Diptera, and Hymenoptera [8]. Because the host insects contain a variety of microorganisms, the sclerotia of *Cordyceps* also contain various microorganisms [9]. In sclerotia of *C. militaris* collected in Yunnan Province, China, the bacteria identified included members of the phyla Proteobacteria, Acidobacteria, Bacteroidetes, and Actinobacteria, and the genera *Pedobacter*,

*Phyllobacterium*, *Pseudomonas*, *Mesorhizobium*, *Bradyrhizobium*, *Variovorax*, *Sphingomonas*, and others [10]. The bacteria in sclerotia of *C. sinensis* were dominated by Proteobacteria and Actinobacteria and included *Pseudomonas*, *Rhodoferax*, *Pedobacter*, and *Sphingomonas* [11, 12]. In the sclerotia of *C. cicadae*, Proteobacteria, Bacteroidetes, and Actinobacteria were the main bacterial groups, and *Pseudomonas* and *Serratia* were dominant genera [13].

Some of these symbiotic/associated microorganisms have the ability to regulate the growth characteristics and metabolites of *Cordyceps*. *Herbaspirillum* and *Phyllobacterium* on the fruiting body can increase the bioactive compound content of *C. militaris* [10]. Three species of bacteria (*Serratia marcescens*, *Cedecea neteri* and *Enterobacter aerogenes*) isolated from *C. cicadae* promoted the production of N6-(2-hydroxyethyl) adenosine and decreased the production of adenosine, uridine and guanosine [14]. In co-culture, the color of the fungus and the morphology of its mycelia may change [15, 16]. The effect of microorganisms on the sclerotia of *C. militaris* needs further research.

In this study, the microbial composition in the sclerotia of wild *C. militaris* from Liaoning Province, China, was analyzed using high-throughput sequencing technology, and two strains of bacteria were isolated, characterized, and identified. Then, these strains were co-cultured with *C. militaris* to explore the effects on mycelium pellet morphology, biomass of mycelium pellets, and production of cordycepin and polysaccharides.

## Results And Analysis

### Bacterial community composition and ecological function

A total of 204,067 effective sequences were detected in three samples of *C. militaris* collected in October 2019 in Liaoning Province, China; 62,929–71,212 sequences were obtained for each sample, with average length 423.45–425.61 bp. With the increase of the number of sample sequences, the Shannon-Winner index curve flattened out (Additional file 1: Table S1), indicating that the sequencing data depth in this experiment comprehensively reflected the bacterial community in the samples.

Bacteria identified in the sclerotia included 21 phyla, 46 classes, 123 orders, 195 families, 321 genera, 450 species, and 549 operational taxonomic units (OTUs). At the phylum level (Fig. 1), Proteobacteria (average relative abundance of OTU 68%), Bacteroidetes (24%), and Actinobacteria (8%) were dominant. At the genus level, *Pseudomonas* (17%), Unclassified Enterobacteriaceae (14%), *Pedobacter* (11%), *Sphingobacterium* (11%), *Serratia* (10%), *Rhodococcus* (6%), and *Acromobacter* (6%) were dominant.

Forty-two OTUs were common to the three samples (Additional file 2: Fig. S2), accounting for only 7.65% of the total number of OTUs. These 42 OTUs were uploaded to the FAPROTAX system [17] for analysis, and predicted functions of 17 genera represented by 21 OTUs were identified (Fig. 2; Additional file 5: Table S1). *Stenotrophomonas* (OTU1490) is animal parasitic or symbiotic, and is a human pathogen. It actively participates in the nitrogen cycle. *Sphingobacterium* (OTU2342) is involved in urea

decomposition. *Pseudomonas* (OTU1448, OTU2330, and OTU2314) is chemoheterotrophic. *Rhodococcus* (OTU1539 and OTU1423) degrades aromatic hydrocarbons and aliphatic non-paraffin hydrocarbons.

### Isolation and identification of bacteria

Two pure strains of bacteria, N-2 and N-26, were isolated from sclerotia of *C. militaris*. Strain N-2 is short rod-shaped ( $7.2\text{--}9.4 \times 3.2\text{--}3.8 \mu\text{m}$ ) (Fig. 3) and Gram-negative; colonies were slightly convex, pale yellow, smooth, moist, and opaque. Physiological and biochemical tests (Additional file 6: Table S2) showed that strain N-2 has swimming ability, can decompose glucose to produce pyruvate, and can decarboxylate the pyruvate and convert it into alcohol and other substances. In addition, ornithine decarboxylase, lysine decarboxylase and amino acid decarboxylase were detected, indicating that strain N-2 can decarboxylate amino acids ( $-\text{COOH}$ ) to produce an amine and  $\text{CO}_2$ . Strain N-2 cannot use mannitol, inositol, sorbitol, melibiose, ribitol, raffinose, xylose, or maltose as carbon sources. Using the methods described in the eighth edition of "*Bergey's Manual of Systematic Bacteriology*", strain N-2 was identified as belonging to the genus *Stenotrophomonas* [18]. By Basic Local Alignment Search Tool (BLAST) analysis, the 16S rRNA gene sequence of strain N-2 was found to be 99.93% identical to that of *S. maltophilia* GZUIFR-YC01. Strain N-2 was identified as *S. maltophilia* (Hugh) (Additional file 4: Fig. S4) (NCBI accession number: MW829549).

Strain N-26 is short rod-shaped ( $9.5\text{--}11.5 \times 4.2\text{--}5 \mu\text{m}$ ), Gram-negative, and its colonies are yellow, smooth, moist and opaque, with a central bulge. The semi-solid agar (dynamic test) of strain N-26 was positive, the Voges-Proskauer test was positive, and the Methyl Red test was negative. The strain was positive for ornithine decarboxylase, lysine decarboxylase and amino acid decarboxylase. The strain could not use mannitol, inositol, sorbitol, melibiose alcohol, raffinose, xylose, or maltose as carbon sources. In BLAST analysis, the 16S rRNA gene sequence of strain N-26 was 99.71% identical to that of *Pseudomonas baetica* YHNG5 (Additional file 4: Fig. S4), which led to the identification of strain N-26 as *P. baetica* (Lopez) (NCBI Accession number: MW829550).

### Interaction between isolated bacteria and *C. militaris*

On potato-dextrose-agar (PDA) plates, *S. maltophilia* N-2 had an inhibitory effect on the growth of mycelium of *C. militaris*. Strain N2 released something that slowed the growth of *C. militaris* mycelia near the area of *S. maltophilia* N-2 growth (Fig. 4a). *P. baetica* N-26 did not inhibit mycelial growth on PDA plates (Fig. 4b).

The dry weight of mycelium pellets decreased from 1.57 g/flask in the control to 0.21 g/flask in the presence of *S. maltophilia* strain N-2 or 0.35 g/flask in the presence of *P. baetica* strain N-26 strain after co-culture with *C. militaris* for 7 days; these differences were highly significant when compared with the control ( $P < 0.01$ ) (Fig. 5a). The diameter of mycelium pellets decreased from 7.38 mm in the control to 3.29 and 3.63 mm after culture in the presence of strains N-2 and N-26, respectively ( $P < 0.01$ ) (Fig. 5b). The addition of bacteria decreased the cordycepin content of the culture medium from 44.04  $\mu\text{g/mL}$  in the control to 6.55  $\mu\text{g/mL}$  with strain N-2 or 2.69  $\mu\text{g/mL}$  with strain N-26 ( $P < 0.01$ ) (Fig. 5c). The content

of extracellular polysaccharide (EPS) was decreased (5.08 mg/mL) after N-2 supplementation, and the difference was significant compared with the control (6.75 mg/mL) ( $P < 0.05$ ), while the addition of strain N-26 increased the amount of EPS (7.38 mg/mL) (Fig. 5d).

## Discussion

The bacteria present in sclerotia of *C. militaris* sampled from Liaoning Province, China, were identified using high-throughput sequencing technology. *Pseudomonas* were more abundant in sclerotia of *C. militaris* isolated in Liaoning Province than in *C. militaris* isolated in Yunnan Province, while *Phyllobacterium*, *Mesorhizobium*, and *Bradyrhizobium* were less abundant in the former [10]. The relative abundance of *Mesorhizobium*, *Bradyrhizobium*, *Sphingomonas*, and *Labrys* in sclerotia samples from Liaoning Province was lower than in samples from Yunnan Province (Table 1). *Pseudomonas*, *Pedobacter*, *Phyllobacterium*, *Mesorhizobium*, *Bradyrhizobium*, *Sphingomonas*, *Variovorax*, and *Labrys* were found in *C. militaris* samples from both Yunnan Province (southwest China, 25.40 N, 102.92 W) and Liaoning Province (northeast China, 42.39 N, 124.26 E), but their relative abundances were different, which may be related to differences of the insect host and environmental conditions [19]. These same bacteria found in sclerotia of *C. militaris* from different geographical locations may be the key microorganism in the microenvironment of *C. militaris* and perform important functions [20]. Functional prediction (Table 1) showed that *Pseudomonas*, *Pedobacter*, *Labrys*, and *Sphingomonas* are chemoheterotrophic, while *Stenotrophomonas* functions in the nitrogen cycle. *Pseudomonas*, *Phyllobacterium*, *Mesorhizobium*, *Bradyrhizobium*, *Pedobacter*, *Variovorax*, and *Sphingomonas* belong to the microbiome of the plant rhizosphere [21,22]. These microorganisms may help to maintain plant hormone balance, control root development, promote nutrient acquisition, and prevent disease, improving plant growth and maintaining plant health [23]. The microorganisms are also ingested by root-feeding insects. When *Cordyceps* spp. invade insects, the microorganisms in the insect gut interact with the fungus.

*Stenotrophomonas maltophilia*, a parasitic bacterium of insects [24], can promote the digestion and absorption of food by the host by secreting enzymes such as cellulase, amylase, protease, and chitinase; these enzymes inhibit the integrity of fungal hyphae and biofilm formation [25-27]. In insects, *S. maltophilia* can inhibit the growth of *Beauveria bassiana* (a fungus that parasitizes arthropods) [28]. Therefore, *S. maltophilia* can play a protective role in an insect host. *S. maltophilia* participates in the sulfur and nitrogen cycles, degrades complex compounds and pollutants, and promotes plant growth and health [29]. We conclude that *S. maltophilia* plays an important ecological role in the sclerotia of *C. militaris*.

*Pseudomonas* has many functions, e.g., *P. fluorescens* secretes luciferin and inhibits the growth of *Escherichia coli* in insects [30], decomposes wood, synthesizes multiple vitamins, and suppresses fungi in beetles [31]. *P. aeruginosa* strain BGF-2 isolated from German cockroach could inhibit the growth of *B. bassiana* [32]. *Pseudomonas* has a flexible metabolism that allows it to synthesize a wide range of

antibiotics to ward off competitors and protect itself from predators, and to produce chemical signaling molecules that sustain intraspecies and interspecies interactions [33].

The relative abundance of bacteria in insects may be related to growth stimulation by parasitic fungi. For example, the biomass of *P. fragi* (a bacterium found in *Thitarodes* and *Hepialus* ghost moths) increased after invasion by *C. sinensis*, and *P. fragi* became the dominant bacterium and participated in the process of larval mummification [34]. Both *Stenotrophomonas* and *Pseudomonas* have been reported to inhibit conidial germination and mycelial growth of *B. bassiana* [28]. The two bacterial strains tested in this study had a similar effect on the hyphae of *C. militaris*. These findings indicate that using parasitic bacteria to inhibit fungal invasion is a protection mechanism of insects.

In the present study, the biomass of *C. militaris* decreased after co-culture with strain N-2 (*S. maltophilia*) or N-26 (*P. baetica*), so the content of cordycepin in the culture medium decreased accordingly. Several mechanisms could explain this effect. One is inhibition of the expression of cordycepin-synthesis-related genes (*cns1*, *cns2*, *cns3*, and *cns4*) [35]; this can be verified by quantitative PCR in later study. Alternatively, (a) key enzyme(s) involved in cordycepin synthesis may have been inhibited. It is also possible that the mycelium structure was damaged in the co-culture process, which could be observed by using cryo-electron microscopy after co-culture. Or, cordycepin may be produced at around the usual level but used by the co-cultured bacterium. Why the polysaccharide content in cultures treated with strain N-26 was higher than that in the control group also requires further study.

Because cordycepin has antibacterial function [36], we speculate that its ecological role is to inhibit the growth of some bacteria, to create good conditions for *C. militaris* and keep the insect host from decaying. Our data indicate that some symbiotic bacteria may inhibit the production of cordycepin. We speculate that other symbiotic bacteria may promote the production of cordycepin. Thus, we aim to isolate other microorganisms from the sclerotia and further explore the relationships between the isolates and *C. militaris*. When more and more interactions are revealed, the ecological functions of microbes in the sclerotia will become clear.

## Conclusions

There are diverse bacteria in the sclerotia of *C. militaris*, among which *Pseudomonas*, *Pedobacter*, and *Serratia* are the dominant genera. This study reveals the interactions between *C. militaris* and isolated strains of *Stenotrophomonas maltophilia* and *Pseudomonas baetica*; these bacteria had inhibitory effects on the biomass, mycelial pellet diameter, and cordycepin yield of *C. militaris*.

## Materials And Methods

### Sample source and treatment

Samples: *C. militaris* was collected in October 2019 from Tieling City, Liaoning Province, China, (42.39 N, 124.26 E), 240 m above sea level, in a broad-leaved mixed forest. The strain *C. militaris* (L.) Link was

stored in the Institute of Fungi Resources of Guizhou University (GZUIFR), strain number SYCM1910.

The 12 *C. militaris* obtained from Liaoning Province were divided into three samples for analysis in this work. In other words, each sample was a mixed sample.

Sclerotia sample preparation followed the method of Zeng [37] with slight modifications. *C. militaris* was rinsed with sterile water to remove residual soil on the insect surface, soaked alternatively with 75% alcohol and 2% sodium hypochlorite three times for 20 s each time, and then rinsed with sterile water. After removal of the body wall of the insect, the specimen was sclerotia. Each sclerotia sample of *C. militaris* weighed about 3.5 g. The samples were stored at  $-80\text{ }^{\circ}\text{C}$  until analysis.

### **Culture media and isolation of bacterial strains**

LB medium contained tryptone 10 g/L, yeast extract 5 g/L, NaCl 10 g/L, and agar 15 g/L (pH  $\approx$  7.0). Potato-dextrose-agar contained potato 200 g/L, glucose 20 g/L, and agar 15 g/L (pH  $\approx$  7.0). Sabouraud's medium contained glucose 40 g/L and peptone 10 g/L (pH  $\approx$  7.0).

*C. militaris* sclerotia sample (0.5 g) was ground and its suspension was placed on LB-agar for microbial isolation.

### **Total bacterial DNA extraction, PCR amplification, and high-throughput sequencing**

*C. militaris* sclerotia samples (3.0 g) were taken, ground in liquid nitrogen, and total microbial DNA was extracted according to the instructions of the E.Z.N.A.® SOIL DNA Kit (Omega, USA). PCR amplification used TransStart FastPFU DNA Polymerase. The reaction system contained: 5 $\times$  FastPFU buffer (4  $\mu$ L), 2.5 mmol/L dNTPs (2  $\mu$ L), 5  $\mu$ mol/L primers 338F (5'-ACTCCTACGGGAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTA-3') (0.8  $\mu$ L each) targeting the V3–V4 region of 16S rRNA genes [38], FastPFU Polymerase (0.4  $\mu$ L), bovine serum albumin (0.2  $\mu$ L, 1  $\mu$ g/ $\mu$ L), and template DNA (10 ng), supplemented with ddH<sub>2</sub>O to 20  $\mu$ L. Reaction used an ABI Gene AMP® 9700 PCR instrument. The reaction parameters were 95  $^{\circ}\text{C}$  for 3 min; 30 cycles of 95  $^{\circ}\text{C}$  for 30 s, 55  $^{\circ}\text{C}$  for 30 s, and 72  $^{\circ}\text{C}$  for 45 s; and a final extension at 72  $^{\circ}\text{C}$  for 10 min. The amplified products were sent to Shanghai Major Biomedical Technology Co., Ltd. and sequenced using the Illumina MiSeq platform.

### **Identification of bacteria isolated from sclerotia**

Isolated bacteria were observed by scanning electron microscopy (SU8100, HITACHI), and their physiological and biochemical characteristics were identified using bacterial biochemical identification strips HBIG05 and HBIG08 (Qingdao Hopebio Biotechnology Co., Ltd.). Bacterial DNA was extracted according to the procedures for the Bacterial Genomic DNA Extraction Kit DP2002 (Beijing Biotek Biotechnology Co., Ltd.). The 16S rRNA gene was amplified in all DNA extracts using primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTACGACTT-3') [39]. The reaction mixture (25  $\mu$ L in total) contained 1  $\mu$ mol/L primers (1  $\mu$ L each), 10 ng/ $\mu$ L DNA template (2  $\mu$ L), Master Mix (green) (including DNA polymerase, buffer, and dNTPs; 12.5  $\mu$ L, product number TSE005, Tsingke

Biotechnology Co., Ltd.) and ddH<sub>2</sub>O (8.5 μL). The PCR conditions were: pre-denaturation at 95 °C for 3 min; 32 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s; and a final extension at 72 °C for 10 min. PCR products were sequenced by Tsingke Biotechnology Co., Ltd. Using the sequence data, BLAST was performed against DNA sequences in GenBank, and the sequences of related species (similarity >97%) were downloaded. Using *Pseudomonas carboxydohydrogena* as the outgroup, a phylogenetic tree was constructed by the neighbor-joining method using MEGA X software with 1000 bootstrap replicates.

## Functional analysis of bacteria isolated from sclerotia

### Plate co-culture

*C. militaris* SYCM1910 was inoculated in the center of a PDA plate and cultured at 25 °C for 7 days. Then, bacteria were inoculated at three locations on the periphery of the colony (25 mm from the point where *C. militaris* had been inoculated) and culture was continued at 25 °C for 7 days.

### Coculture in liquid

A piece of mycelium block of *C. militaris* cultured for 21 days was inoculated into a 250-mL triangular flask containing 100 mL Sabouraud's medium and cultured on a magnetic stirrer at 25 °C for 3 days. Then, 1 mL/flask of bacterial suspension (bacterial cell density  $1.5 \times 10^8$  colony-forming units/mL) was added and culture was continued at 120 r/min and 25 °C for 7 days.

Measurement of physical indicators: Dry weight of mycelium pellets was determined after the fermentation broth was filtered using qualitative filter paper and the pellets were dried to constant weight at 60 °C. Then, the mycelium pellet diameter was measured using vernier calipers.

Biochemical determination: Cordycepin was determined by high-performance liquid chromatography according to the Agricultural Industry Standard NY/T 2116-2012 of the People's Republic of China, using a Thermo Fisher Ultima 3000RS system and a C<sub>18</sub> column with mobile phase acetonitrile : water (5:95 v:v) at flow rate 1.0 mL/min, column temperature 35 °C, detection wavelength 260 nm, and sample volume 10 μL. EPS was determined by the anthrone sulfuric acid method [40].

## Data processing

### Sequence processing and OTU annotation

Paired-end reads obtained by MiSeq sequencing were first stitched according to overlap relationship, and sequence quality was controlled and filtered at the same time. Effective sequences were obtained by distinguishing samples according to barcode and primer sequences at both ends of the sequence, and sequence direction was corrected to obtain optimized sequences. Using UPARSE software (<http://www.drive5.com/uparse/>), repetitive sequence OTU clustering was carried out with a threshold of

97% similarity, chimeras were removed in the process of clustering, and the RDP database (<http://rdp.cme.msu.edu/>) was used for OTU annotation.

The raw sequence reads obtained in this study were deposited in the NCBI Sequence Read Archive database under accession number PRJNA722375.

### **Prediction of bacterial function**

FAPROTAX (<http://www.zoology.ubc.ca/louca/FAPROTAX/>) is a tool that can predict ecological functions of bacterial and archaea taxa from 16S rRNA amplicon sequencing [17]. We used it to identify ecological functions of OTUs. An OTU abundance table and taxonomic annotation of OTUs were inputted in the corresponding option box, and the PLOT option was selected. Then, predicted function output was obtained as an Excel spreadsheet. A heatmap was generated using the online tool at the website [www.ehbio.com/ImageGP/](http://www.ehbio.com/ImageGP/).

### **Data analysis and presentation**

Statistical analysis of the experimental data was performed using SPSS software v.22.0. The least significant difference test was used for one-way analysis of variance.

## **Abbreviations**

BLAST: Basic local alignment search tool; NCBI: National Center for Biotechnology Information; EPS: Extracellular polysaccharide; ddH<sub>2</sub>O: Double distilled water.

## **Declarations**

### **Authors' contributions**

Li Luo conducted the experiments and wrote the manuscript. Jia-Xi Zhou was in charge of the revision of the manuscript. Zhong-Shun Xu provided samples. Ying-Ming Gao prepared the figures. Xiao Zou was in charge of reviewing the manuscript and funding the experiments.

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### **Availability of data and materials**

All data generated or analyzed during this study are included in this published article and its Supplementary Information files.

## Ethics approval and consent to participate

Not applicable.

## Consent for publication

Not applicable.

## Competing Interests

The authors declare that they have no competing interests.

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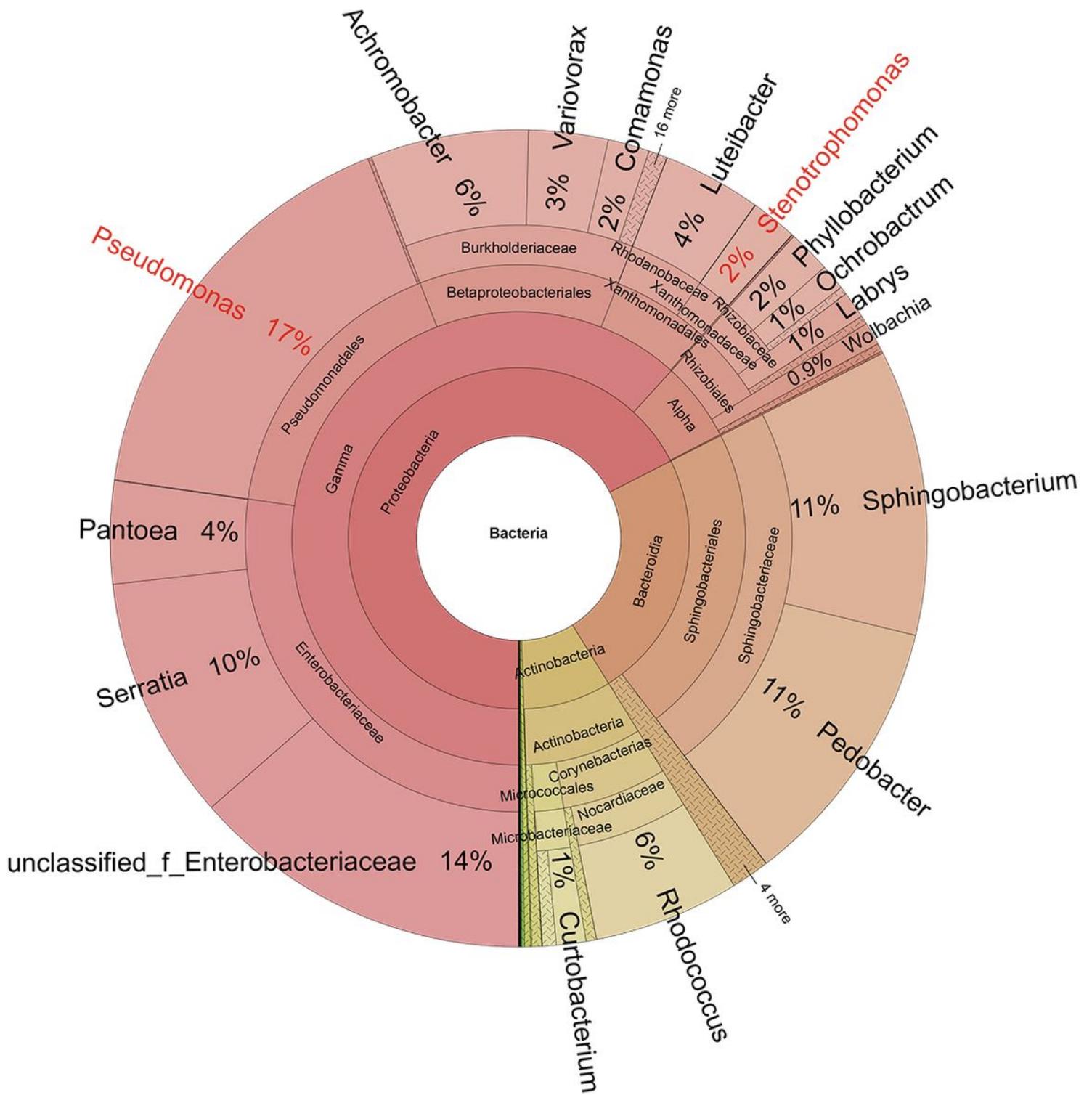
## Tables

**Table 1** Relative abundance and function prediction of bacterial composition in sclerotia of *C. militaris* isolates.

Genus	<i>C. militaris</i> from Yunnan	<i>C. militaris</i> from Liaoning	Predicted Function
<i>Pseudomonas</i>	2.01%-15.00%	16.68%	aerobic chemoheterotrophy☒ chemoheterotrophy☒
<i>Pedobacter</i>	2.01%-15.00%	10.81%	aerobic chemoheterotrophy☒ chemoheterotrophy☒
<i>Variovorax</i>	2.01%-15.00%	3.14%	None
<i>Phyllobacterium</i>	2.01%-15.00%	1.69%	None
<i>Labrys</i>	2.01%-15.00%	0.98%	aerobic chemoheterotrophy☒chemoheterotrophy☒
<i>Mesorhizobium</i>	2.01%-15.00%	0.05%	nitrogen fixation☒aerobic chemoheterotrophy☒ ureolysis☒chemoheterotrophy☒
<i>Sphingomonas</i>	2.01%-15.00%	0.06%	aerobic chemoheterotrophy☒chemoheterotrophy☒
<i>Bradyrhizobium</i>	2.01%-15.00%	0.02%	None
<i>Serratia</i>	---	9.65%	fermentation☒ nitrate reduction☒ chemoheterotrophy☒plant pathogen☒
<i>Achromobacter</i>	---	6.26%	aerobic chemoheterotrophy☒ nitrate respiration☒ nitrate reduction☒nitrogen respiration☒ chemoheterotrophy☒
<i>Rhodococcus</i>	---	5.78%	aromatic hydrocarbon degradation☒aromatic compound degradation☒aliphatic non methane hydrocarbon degradation☒hydrocarbon degradation☒chemoheterotrophy☒ ligninolysis☒ plant pathogen☒
<i>Pantoea</i>	---	4.06%	fermentation☒mammal gut☒animal parasites or symbionts ☒nitrate reduction☒ chemoheterotrophy☒
<i>Luteibacter</i>	---	3.89%	None
<i>Stenotrophomonas</i>	---	1.82%	nitrate respiration☒ nitrate reduction☒ nitrogen respiration☒aerobic chemoheterotrophy☒human pathogens☒ animal parasites or symbionts ☒chemoheterotrophy☒
<i>Ochrobactrum</i>	---	1.02%	None

Note: “-” means not mentioned in the literature; “None” means that there was no result when a function was predicted using FAPROTAX.

## Figures



**Figure 1**

Taxonomic composition of the microbiome in sclerotia of *Cordyceps militaris*. Circles from inside to outside represent the community composition of the bacteria at different classification levels (kingdom, phylum, class, order, family, and genus, respectively). The size of the fan segments represents the relative proportion of the annotation results of different bacterial OTUs.

# Main\_Title

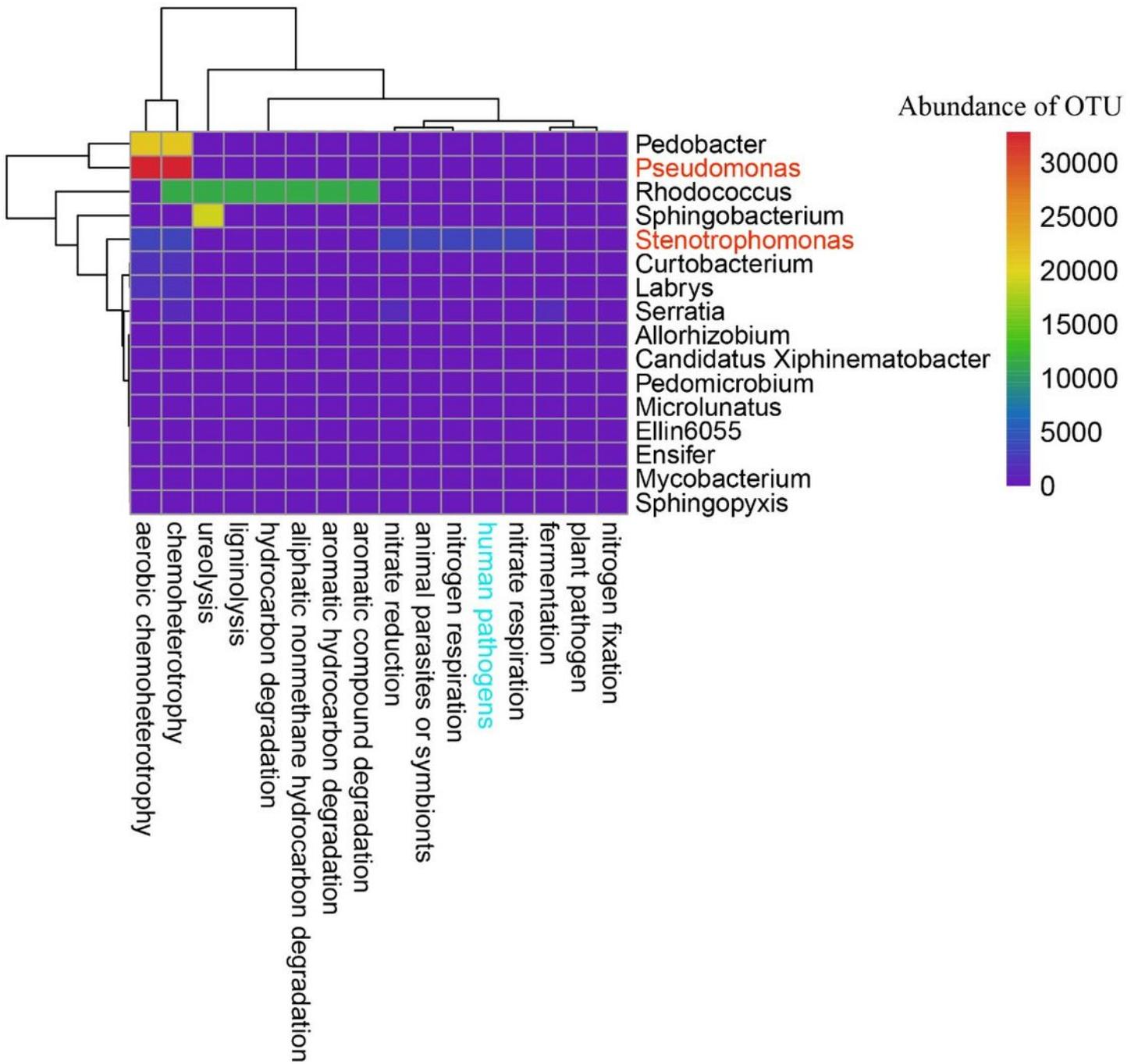
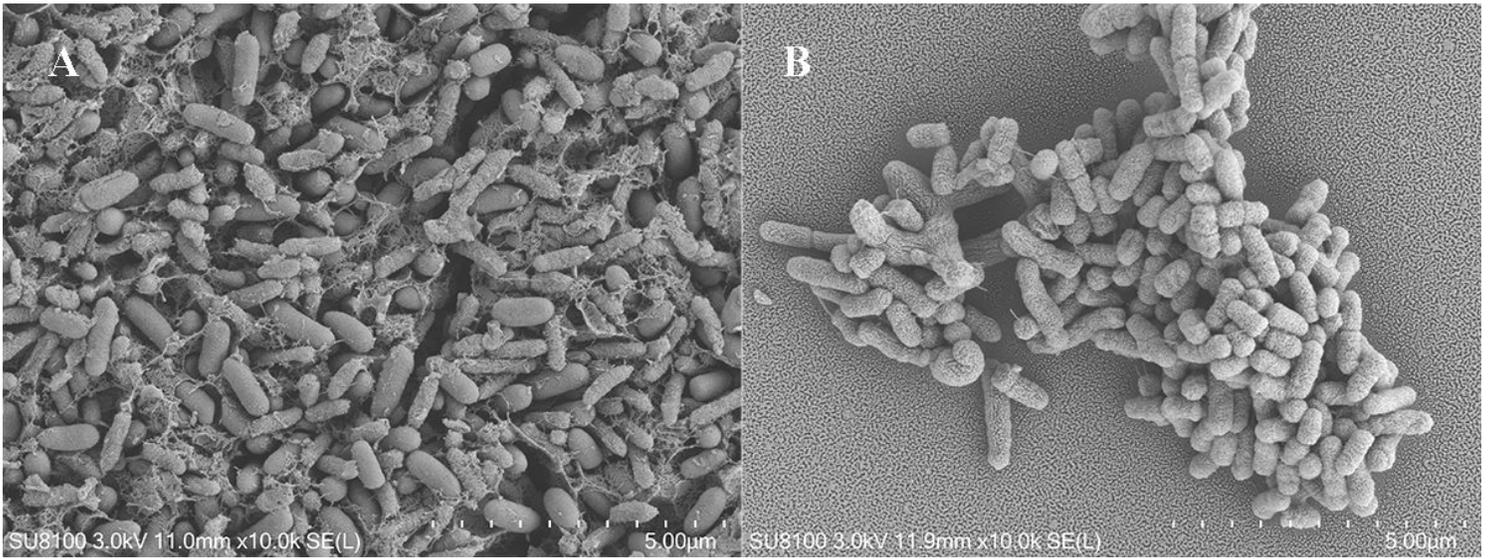


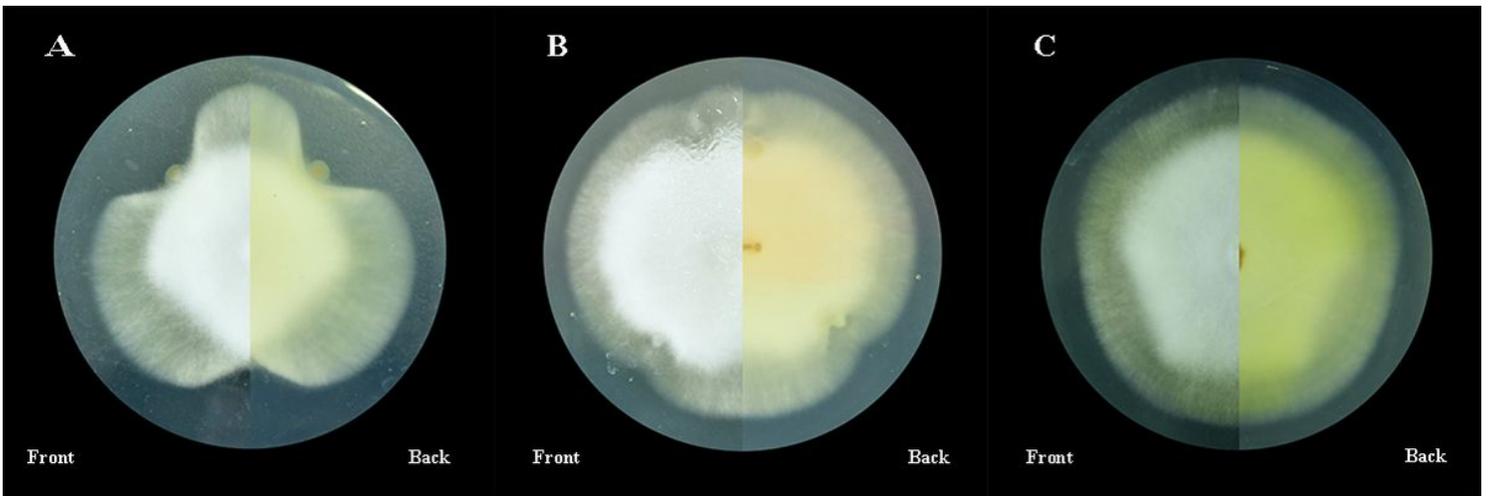
Figure 2

Functional prediction of the bacterial core microbiome in sclerotia of *C. militaris* using FAPROTAX. Genera in red are the subject of this study.



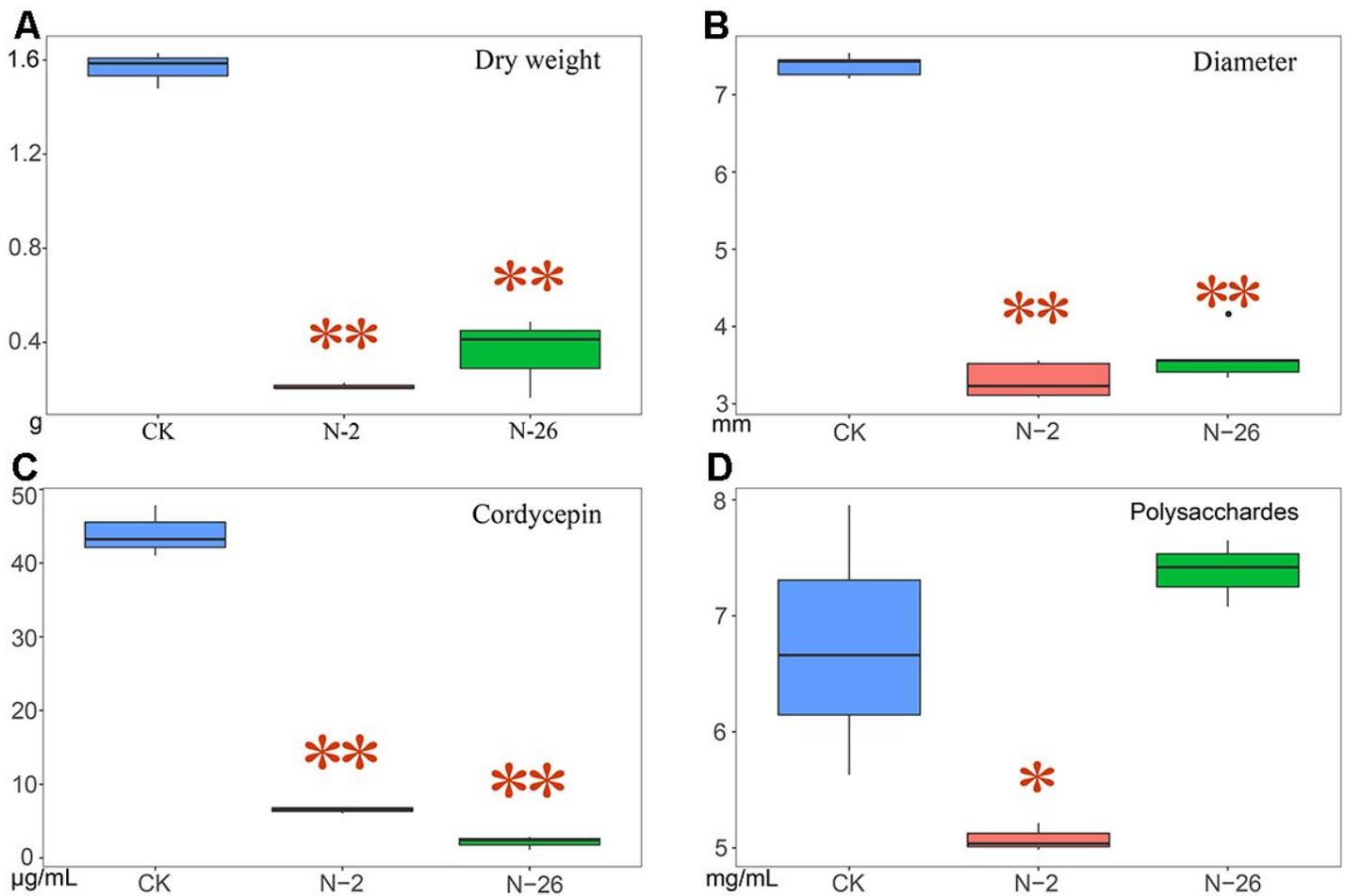
**Figure 3**

Scanning electron micrographs of strains a N-2 and b N-26. Note: In Fig. 3a, the reticular-like structure on the surface of the bacteria is secretion by the bacteria. Note: In Fig.3a, the reticular structure on the surface of bacteria is the secretion of bacteria itself.



**Figure 4**

Co-culture on potato-dextrose-agar. a Strain N-2 and *C. militaris*, b strain N-26 and *C. militaris*, c *C. militaris* only.



**Figure 5**

Effects of coculture of isolated bacterial strains with *C. militaris*. a Dry weight of mycelium pellets. b Diameter of mycelium pellets. c Cordycepin concentration in medium. d Polysaccharide concentration in medium. \*P < 0.05, \*\*P < 0.01.

## Supplementary Files

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