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# Isolation, identification and antagonistic activity evaluation of actinomycetes in barks of nine trees

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**Abstract:** Actinomycetes are important producers of novel bioactive compounds. New sources need to be explored for isolating previously unknown bioactive compound-producing actinomycetes. Here we evaluated the potential of bark as a natural source of novel bioactive actinomycete species. Bark samples were collected from nine tree species at different elevations (1600-3400 m a.s.l.) on Qin Mountain, Shaanxi Province, China. Actinomycetes were cultivated, enumerated and isolated using serial dilution and spread-plate techniques. The antimicrobial activity of actinomycete isolates was analyzed using an agar block method against 15 typical bacterial and fungal species and plant pathogens. The dominant isolates were identified by 16S rRNA-based sequence analysis. Results showed that actinomycete counts in bark samples of *Quercus liaotungensis* Koidz. was the highest among all trees species tested. The numbers of actinomycete species in bark samples were highest in *Q. aliena* var. *acutiserrata* and *Spiraea alpina* Pall. Antagonistic activity was detected in approximately 54% of the actinomycete isolates. Of these, 20 isolates (25%) showed broad-spectrum antagonistic activity against ≥5 of the microorganisms tested. In conclusion, the bark on coniferous and broadleaf trees possesses a high diversity of actinomycetes and serves as a natural source of bioactive compound-producing actinomycetes.

**Key words:** antagonistic potentiality; bioactive compound; Gause's synthetic agar; modified humid acid agar; Qin Mountain

## INTRODUCTION

Approximately 45% of bioactive microbial metabolites are produced by actinomycetes [1]. Isolation of novel bioactive compound-producing actinomycetes is fundamental to research and the development of new drugs urgently needed for human health and agriculture. Actinomycetes are widely distributed in natural ecosystems. Extensive studies have explored soils, sediments and water bodies from which numerous bioactive actinomycete species were isolated. Representatives are *Streptomyces* and *Micromonospora* [1]. In recent decades, it has become increasingly difficult to find novel bioactive actinomycetes in the above natural ecosystems. In this context, alternative ecosystems, especially the internal and external environments of plants, have attracted substantial attention of researchers [2-7].

Over the past few decades, it has been recognized that microorganisms of enormous diversity are residing in plant tissues, including tree bark [8]. Several studies have investigated the actinomycete populations in tree barks during composting. The highest abundance of actinomycete population is detected in the thermophilic phase (>40°C) of bark composting [9]. Additionally, antagonistic endophytic actinomycetes have been isolated from the compost barks of sugar apple (*Annonaceae squamosal*), cancer tree (*Camptotheca acuminate*), Chinese yew (*Taxus chinensis*) and eucalyptus (*Eucalyptus globulus*) [10,11].

At present, knowledge is lacking regarding endophytic actinomycetes in the bark of woody plants. Bark in the older stems of trees includes dead tissues on the surface of stems along with parts of the innermost periderm and all the tissues on the outer side of the periderm [12]. In the bark of trees, dead tissues

provide nutritive substances and irregular precipitation is the source of water for microbial activities. In this way, the bark of trees could satisfy the growth requirements of actinomycetes, providing conditions for the colonization with optimal numbers of endophytic actinomycetes. Recently, Kitouni et al. [13] isolated 10 actinomycete isolates from oak and cedar barks, of which 4 isolates were found to be antagonistic to typical bacteria. However, no research has specifically analyzed the characteristics of actinomycetes in bark of typical trees on Qin Mountain.

To this end, the present study investigated actinomycete populations residing in the barks of nine species of coniferous and broadleaf trees on Qin Mountain. We evaluated the potential for isolating novel bioactive compound-producing actinomycetes from the special ecosystem of bark by a colony count of actinomycetes, determination of the total number of actinomycetes species and identification of the dominant actinomycete species, followed by an evaluation of their antagonistic activity. The results were analyzed to explore the distribution of antagonistic actinomycetes in the bark of woody trees so as to facilitate actinomycete screening for novel bioactive-compound producers from natural resources.

## MATERIALS AND METHODS

### Study site and bark sampling

This study was carried out on Qin Mountain (33°57′-34°58′N, 107°45′-107°53′E), Shaanxi Province, China. Qin Mountain is a natural geographic and climatic boundary between north and south China. There are five climatic belts, from warm temperate to alpine frigid zone, and seven altitudinal belts of vegetation on Qin Mountain. Nine typical species of trees, including four coniferous species and five broadleaf species, were selected from the seven vegetation belts. The coniferous species included *Larix chinensis* Beissn. (LB), *Abies fargesii* Franch. (AF), *Larix chinensis Beissn.* (LB) and *Abies fargesii* Franch. (AF). The broadleaf species included *Spiraea alpina Pall.* (SP), *Betula albosinensis* var. *septentrionalis* (BS), *B. albosinensis* Burk. (BB), *Quercus liaotungensis* Koidz. (QK) and *Q. aliena* var. *acutiserrata* (QA). For LB, bark samples were collected at two elevations, 3165 m (LBH) and 2739 m (LBL). Elevations of other samples are shown in Table 1. The pieces of bark, 1.0 cm in thickness (0.3 cm for SP and BB), were cut off using a sterile blade at 1.0-1.5 m above the ground (0.5 m for SP). The samples were sealed in sterile polyethylene bags, transported to the laboratory, and stored in the dark at 4°C until use.

### Actinomycete cultivation, enumeration and isolation

Serial dilution and spread-plate techniques [14] were used to isolate actinomycetes from bark samples. The samples were surface-sterilized by dipping in 70% ethanol and 0.1% mercuric chloride, each for 1 min. Serial dilutions were prepared by adding 5 g of grinded bark to 45 mL of sterile distilled water ($10^{-1}$) in a conical flask, followed by oscillation at 160 rpm for 10 min. and further dilution to $10^{-5}$. The dilutions of $10^{-3}$ to $10^{-5}$ were used. Two agar media were tested: Gause's synthetic agar (20 g soluble starch, 1 g $KNO_3$, 0.5 g $K_2HPO_4$, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.5 g NaCl, 0.01 g

**Table 1.** Sources and chemical properties of samples.

| Tree types | Tree species | Samples | Elevation (m) | pH | Key elements (g kg$^{-1}$) | | |
|---|---|---|---|---|---|---|---|
| | | | | | N | K | P |
| Coniferous | *Larix chinensis* Beissn. | LBH | 3165 | 6.07 | 10.3 | 24.9 | 2.6 |
| | *Abies fargesii* Franch. | AF | 2765 | 6.30 | 6.6 | 11.8 | 1.5 |
| | *L. chinensis* Beissn. | LBL | 2739 | 5.11 | 9.4 | 27.8 | 3.6 |
| | *Pinus armandii* Franch. | PF | 2623 | 6.22 | 7.0 | 9.4 | 1.6 |
| | *P. tabulaeformis* Carr. | PC | 1917 | 4.50 | 2.0 | 1.5 | 0.3 |
| Broadleaf | *Spiraea alpina* Pall. | SP | 3419 | 6.24 | 7.3 | 8.9 | 1.6 |
| | *Betula albosinensis* var. *septentrionalis* | BS | 2614 | 5.73 | 7.1 | 10.2 | 2.3 |
| | *B. albosinensis* Burk. | BB | 2252 | 6.62 | 5.9 | 3.9 | 1.8 |
| | *Quercus liaotungensis* Koidz. | QK | 1917 | 7.00 | 7.7 | 11.4 | 2.0 |
| | *Q. aliena* var. *acutiserrata* | QA | 1600 | 6.83 | 8.8 | 5.8 | 1.4 |

$FeSO_4$, 10 g agar, 1000 mL distilled water) and modified humic acid agar (10 g humic acid, 0.5 g $Na_2HPO_4$, 1 g KCl, 0.05 g; $CaCl_2$ 1 g $MgSO_4 \cdot 7H_2O$, 10 g agar, 1000 mL distilled water). All media were supplemented with 80 mg $L^{-1}$ potassium dichromate to inhibit the growth of bacteria and fungi. After inoculation, all plates were incubated at 28°C for 15 days. Actinomycete colonies were identified by visual examination of the cultural and morphological characteristics; microscopic examination was performed if needed. Morphologically distinct colonies were transferred onto Gause's synthetic agar slants separately, incubated at 28°C for 7 days, and then stored in the dark at 4°C. All experiments were performed in triplicate. The average number of actinomycete colonies on each plate was counted. Data are reported as colony-forming-unit (CFU) $g^{-1}$ stove-dry bark. The colony numbers were compared between different tree species by t-test in SAS 9.0 statistical software (SAS Institute Inc., Cary, NC, USA.).

### Actinomycete identification

The dominant actinomycete isolates and isolates with broad-spectrum antagonistic activity were identified by 16S rRNA-based sequence analysis. Actinomycete DNA was extracted from pure isolates using the method described by Saito and Miura [15]. Partial 16S rRNA gene fragments were amplified by polymerase chain reaction (PCR) using the bacterial primers 27F: 5'-AGAGTTTGATCCTGGCTCAG-3' and 1541R: 5'-AAGGAGGTGATCCAGCCGCA-3'. Amplification was carried out in a DNA Engine thermal cycler (BIO-RAD, USA), using a 50-μL reaction mixture containing 4 μL Taq DNA polymerase (2.5 U $\mu L^{-1}$, Genscript, Nanjing), 5 μL 10× buffer (Transgene, Beijing), 1 μL 20 mM deoxynucleoside triphosphate (Transgene), 37μL of sterile distilled water, 1 μL of each primer (50 μM), and 1 μL of template. The PCR thermocycling conditions were as follows: initial denaturation at 94°C for 4 min; 30 cycles at 94°C for 1 min, 56°C for 1 min, 72°C for 2 min; and a final elongation at 72°C for 10 min. PCR reactions were purified and sequenced by Genscript Biotech (Nanjing) Co., Ltd, China. The obtained sequences were compared with available reference sequences in the EMBL/GenBank/DDBJ databases and deposited in GeneBank under the accession Nos. KF447933–KF447962.

### Antimicrobial activity assay

The antimicrobial activity of actinomycete isolates isolated on Gause's synthetic agar was analyzed using an agar block method [16] against 4 bacterial species and 11 fungal species, which were provided by the Microbiology Laboratory in Shanxi Normal University. The bacterial species included *Escherichia coli* E1, *Staphylococcus aureus* S4 and two pathogens of konjac soft rot, *Serratia* sp. H1 and *Dickeya dadantii* subsp. *Dadantii* D3. The fungal species included *Penicillium* sp. P1, *Candida tropicalis* C1 and nine plant pathogens, *Verticillium dahliae* V2, *Fusarium oxysporum* FO1, *F. solani* (Mart.) *Sacc* FSS1, *F. sulphureum* FS1, *F. oxysporum* f. sp. *cucumerinum* FOC1, *F. oxysporum* f. sp. *niveum* FON1, *F. solani* FS3, *F. oxysporum* f. sp. *vasinfectum* FOV1, and *Didymella bryoniae* DB1.

### Antagonistic potentiality assay of bark actinomycetes

The antagonistic potentiality of bark actinomycetes (APBA) was calculated by considering the number and antimicrobial spectrum of actinomycete isolates in the bark ecosystem using the following equation [17]:

$$APBA(\%) = \sum_{1}^{n} T_n \Big/ \sum_{1}^{m}\sum_{1}^{n} T_n \times 100\%$$

where $m$ and $n$ are the numbers of tested bark samples and actinomycete isolates with antagonistic activity, respectively; $T_n$ is the number of target microorganisms to which the actinomycete isolate is antagonistic.

## RESULTS

### Actinomycete colony counts and species number on Gause's synthetic agar

On Gause's synthetic agar, a large number of actinomycetes capable of using starch as the sole carbon and energy source were recovered from the bark samples tested. The colony count of actinomycetes in bark samples of QK was more than 10-fold that of LBH. The average counts of actinomycete in bark samples of broadleaf trees were 10-fold that of coniferous trees (Table 2).

**Table 2.** Colony counts and species number of actinomycetes (103 CFU g-1 dry bark).

| Samples | Gause's synthetic agar | | Modified humic acid agar | |
|---|---|---|---|---|
| | Counts[1] | Species numbers | Counts | Species numbers |
| *Larix chinensis* Beissn. | 53.7±8.0$^{c}$ | 3 | 0.3±0.1$^{f}$ | 1 |
| *Abies fargesii* Franch. | 29.0±11.3$^{cd}$ | 10 | 20.9±4.5$^{f}$ | 10 |
| *L. chinensis* Beissn. | 1.3±0.6$^{d}$ | 9 | 1.5±0.3$^{f}$ | 6 |
| *Pinus armandii* Franch. | 3.4±0.3$^{d}$ | 5 | 19.8±1.8$^{f}$ | 6 |
| *P. tabulaeformis* Carr. | 0.2±0.1$^{d}$ | 2 | 478.0±30.3$^{c}$ | 8 |
| *Spiraea alpina* Pall. | 11.9±0.5$^{d}$ | 14 | 8.2±3.1$^{f}$ | 6 |
| *Betula albosinensis* var. *septentrionalis* | 3.9±0.8$^{d}$ | 11 | 238.9±19.1$^{e}$ | 7 |
| *B. albosinensis* Burk. | 181.3±14.4$^{b}$ | 9 | 576.2±54.0$^{b}$ | 5 |
| *Quercus liaotungensis* Koidz. | 637.8±143.6$^{a}$ | 4 | 982.4±55.9$^{a}$ | 6 |
| *Q. aliena* var. *acutiserrata* | 151.8±14.6$^{b}$ | 12 | 415.8±31.5$^{d}$ | 8 |

Different letters in the same column refer to significant difference at P<0.05 (t-test).

**Table 3.** Taxonomic distribution and origin of dominant actinomycete species.

| Family | Genera | Isolates | Species | | |
|---|---|---|---|---|---|
| | | | Total | Coniferous trees | Broadleaf trees |
| *Streptomycetaceae* | *Streptomyces* | 22 | 14 | 6 | 10 |
| *Pseudonocardiaceae* | *Umezawaea* | 1 | 1 | 1 | 0 |
| *Nocardiopsaceae* | *Nocardiopsis* | 3 | 1 | 1 | 1 |
| *Micromonosporaceae* | *Micromonospora* | 2 | 2 | 1 | 1 |
| | *Actinoplanes* | 2 | 1 | 1 | 0 |
| Total: 4 | 5 | 30 | 19 | 10 | 12 |

The average number of actinomycete species of broadleaf trees was 72.4% higher than coniferous trees with no statistically significant difference ($P$>0.05). For broadleaf trees, species diversity was rich in SP, BS and QA, while it was poor in BB and QK. For coniferous trees, species diversity was rich in AF and LBL, while it was poor in LBH, PF and PC. (Table 2).

## Actinomycete colony counts and species number on modified humic acid agar

Humic acid is a macromolecular organic matter formed by the breakdown and resynthesis of dead plants. Actinomycetes recovered on modified humic acid agar could use humic acid as the sole carbon and energy source. On modified humic acid agar, actinomycete counts in bark samples of broadleaf trees (except for SP) were significantly more than those of coniferous trees (except for PC, $P$<0.05). Geographically, the colony counts of actinomycetes correlated with elevations ($r$=-0.766, $P$<0.01).

Among all tree species, the total number of actinomycete species in bark samples was highest in AF and lowest in LBH. There was no significant difference in the average of the total number of actinomycete species between broadleaf and coniferous trees ($P$<0.05) (Table 2).

## Dominant actinomycete species

A total of 142 actinomycete isolates were obtained from the bark samples on two media. The dominant isolates were classified into 19 species of five genera: *Streptomyces* spp. (73.7%), *Micromonospora* spp. (10.5%), *Nocardiopsis* spp. (5.3%), *Actinoplanes* spp. (5.3%) and *Umezawaea* spp. (5.3%) (Table 3).

## Antagonistic activity of bark actinomycetes

A total of 79 actinomycete isolates were obtained from the bark samples on Gause's synthetic agar. Of these, 43 isolates were found to be antagonistic to at least one of the 15 microorganisms tested. The average number of actinomycete isolates in broadleaf tree barks was 38.9% higher than that in coniferous tree barks, with no statistically significant difference ($P$>0.05). For broadleaf tree barks, with increasing elevation the numbers of antagonistic isolates showed significant decreases followed by significant increases. For conif-

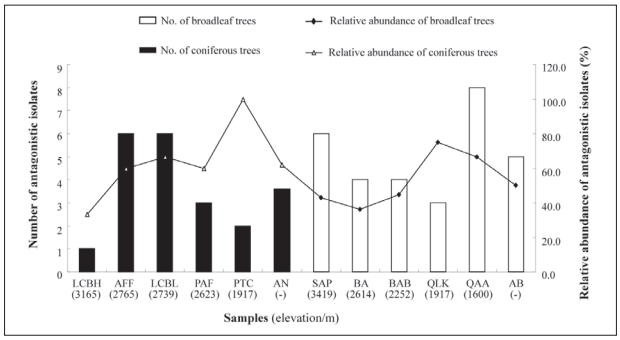


**Fig. 1.** Numbers and relative abundances of antagonistic isolates. AN, AB – the averages of coniferous tree barks and broadleaf tree barks, respectively.

erous tree barks, the numbers of antagonistic isolates in coniferous tree barks first increased and then decreased with increasing elevation (Fig. 1).

The ratio of antagonistic isolates in broadleaf tree barks was 12.1% higher than in coniferous tree barks, with no statistically significant difference ($P$>0.05). With increasing elevation, the ratio of antagonistic isolates substantially decreased in both coniferous and broadleaf tree barks (Fig. 1).

### Antagonistic potentiality

The APBA for coniferous tree barks was lower than for broadleaf tree barks. This indicated that the total reserve of antagonistic actinomycetes was higher in broadleaf tree barks than in coniferous tree barks. APBA substantially varied in the barks of different tree species. For coniferous trees, the APBA in AFF was higher than that in other tree species. For broadleaf trees, the APBA in QAA was higher than in other tree species (Table 4).

### Distribution of antagonistic bark actinomycetes

The number of isolates antagonistic to each target microorganism differed in bark samples between broadleaf and coniferous trees. For 14 out of 15 target microorganisms, the number of antagonistic isolates was 1-7-fold higher in broadleaf trees than in coniferous trees. An exception was *C. tropicalis*, where the number of antagonistic isolates was 5-fold higher in coniferous trees than in broadleaf trees (Table 5).

The numbers of isolates antagonistic to the different target microorganisms varied in bark samples of single tree species. Moreover, the number of isolates antagonistic to each target microorganism varied in bark samples among different tree species. Additionally, the total antagonistic spectra of bark actinomycete isolates substantially varied in different tree species. (Table 5).

### Identification of 5 broad-spectrum antagonistic isolates

The phylogeny and morphology of 5 broad-spectrum antagonistic isolates are shown in Figs. 2 and 3.

## DISCUSSION

In this study, high actinomycete diversity was shown and a large number of actinomycetes with great application potential were residing in the barks of nine different tree species at different elevations on Qin Mountain. These results prove that living tree barks are natural resources with great antagonistic actinomycete reserves, which should receive more attention in research and in the development of new antibiotics and antitumor agents. Additionally, the distribution of antagonistic actinomycetes in barks was dependent on tree species. Even for the same tree species, the distribution of antagonistic bark actinomycetes varied at different elevations. This indicated that regional environmental conditions, especially elevation, could affect the distribution of bioactive compound-producing bark actinomycetes.

**Table 4.** Antagonistic potentiality of actinomycetes in tested barks.

| Antagonistic potentiality | Bark of coniferous trees | | | | | | Bark of broadleaf trees | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | LCBH | AFF | LCBL | PAF | PTC | ∑c [a] | SAP | BA | BAB | QLK | QAA | ∑b | |
| Tn[b] | 7 | 37 | 19 | 15 | 7 | 85 | 14 | 16 | 36 | 14 | 49 | 129 | 214 |
| APBA (%) | 3.3 | 17.3 | 8.9 | 7.0 | 3.3 | 39.7 | 6.5 | 7.5 | 16.8 | 6.5 | 22.9 | 60.3 | 100 |

[a] ∑c – the sum of An or APBA of the 5 coniferous tree barks; ∑b – the sum of An or APBA of the 5 broadleaf tree barks.
[b] $T_n$ – number of target microorganisms to which each isolate could antagonistic; APBA – antagonistic potentiality of bark actinomycetes.

| Samples | | Target microorganisms[a] | | | | | | | | | | | | | | | Σa |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Typical bacteria or fungi | | | | Plant pathogens | | | | | | | | | | | |
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | |
| Bark of coniferous trees | LCBH | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 7 |
| | AFF | 6 | 3 | 3 | 5 | 2 | 0 | 4 | 0 | 3 | 4 | 0 | 3 | 0 | 0 | 3 | 10 |
| | LCBL | 3 | 3 | 1 | 1 | 1 | 1 | 2 | 0 | 2 | 1 | 1 | 1 | 0 | 0 | 2 | 12 |
| | PAF | 3 | 2 | 0 | 3 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 2 | 8 |
| | PTC | 0 | 2 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 6 |
| | ∑c[b] | 12 | 10 | 5 | 11 | 3 | 1 | 9 | 1 | 8 | 7 | 1 | 6 | 0 | 1 | 8 | 14 |
| Bark of broadleaf trees | SAP | 5 | 3 | 0 | 1 | 0 | 2 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 7 |
| | BA | 2 | 1 | 1 | 2 | 1 | 1 | 1 | 0 | 1 | 2 | 1 | 1 | 1 | 0 | 1 | 13 |
| | BAB | 3 | 2 | 3 | 2 | 2 | 0 | 3 | 2 | 3 | 3 | 2 | 3 | 2 | 2 | 4 | 14 |
| | QLK | 3 | 1 | 1 | 0 | 1 | 0 | 2 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 10 |
| | QAA | 6 | 5 | 5 | 1 | 4 | 0 | 6 | 0 | 3 | 5 | 3 | 3 | 1 | 1 | 6 | 13 |
| | ∑b | 19 | 12 | 10 | 6 | 8 | 3 | 13 | 2 | 9 | 11 | 7 | 8 | 4 | 3 | 13 | 15 |
| ∑b-∑c | | 7 | 2 | 5 | -5 | 5 | 2 | 4 | 1 | 1 | 4 | 6 | 2 | 4 | 2 | 3 | 1 |

[a]Target microorganisms: 1 – *Staphylococcus aureus,*2 – *Escherichia coli,* 3 – *Penicillium* sp., 4 – *Candida tropicalis,* 5 – *Serratia* sp., 6 – *Dickeya dadantii* subsp. *dadantii,* 7 – *Verticillium dahliae,* 8 – *Fusarium oxysporum,* 9 – *Fusarium solani(Mart.)Sacc.,* 10 – *Fusarium sulphureum,* 11 – *Fusarium oxysporum* f. sp. *cucumerinum,* 12 – *Fusarium oxysporum* f. sp. *niveum,* 13 – *Fusarium solani,* 14 – *Fusarium oxysporum* f. sp. *vasinfectum,*15 – *Didymella bryoniae*.

[b]∑c and ∑b – the sum of antagonistic isolates in coniferous and broadleaf trees, respectively; ∑a – the number of target microorganisms which all the isolates in each bark sample inhibited.

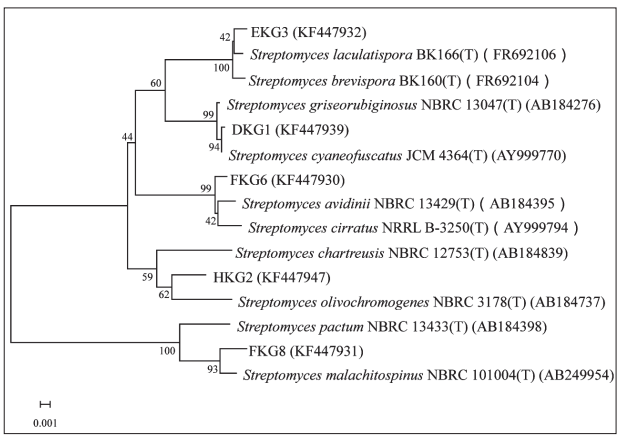


**Fig. 2.** Phylogenetic tree of the 5 broad-spectrum antagonistic isolates.

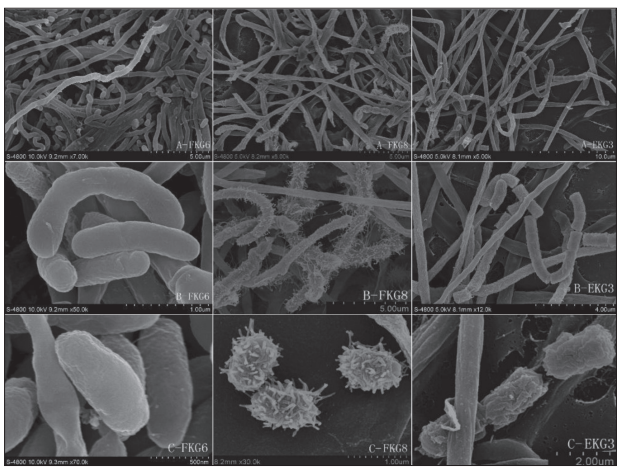


**Fig. 3.** SEM morphology of 3 broad-spectrum antagonistic isolates. A, B and C before the "-" represent mycelium, spore chain and spores, respectively; letters after the "-" represent the names of isolates.

We obtained 5 broad-spectrum antagonistic actinomycete isolates that inhibited the growth of ≥10 target microorganisms. These were identified as *Streptomyces avidinii*, *S. malachitospinus*, *S. laculatispora*, *S. cyaneofuscatus* and *S. olivochromogenes*, respectively. Previously, *S. cyaneofuscatus* [18] and *S. olivochromogenes* [19] were found to produce valinomycin and phospholipase, respectively. To our knowledge, our work provides the first evidence regarding the antagonistic activity of *S. avidinii*, *S. malachitospinus* and *S. laculatispora*. These potentially novel strains of streptomycetes provide alternative resources for research and the development of new antibiotics. Further study needs to identify and characterize their bioactive compounds, especially those from the *S. avidinii*-, *S. malachitospinus*- and *S. laculatispora*-related isolates.

Previous studies have proven that composted bark suppresses specific soil-borne diseases of plants, including root rot, collar rot and some other diseases caused by *Phytophthora* spp., *Pythium* spp. and *Fusarium wilt* [20-22]. In the present study, 54.4% of the

actinomycete isolates showed antagonistic activity. Accordingly, it is concluded that the antagonistic actinomycetes in living tree barks propagated and produced bioactive compounds during the composting process. This may be one of the mechanisms of suppression activity of bark composts against plant pathogens.

In conclusion, a large number of bioactive compound-producing actinomycetes remain unexplored in the barks of living trees. These microbial resources should receive more attention in research and for the development for new antibiotics, antitumor and antiplant pathogen agents.

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