Antimicrobial Activity and Identification of Actinomycete Strains from a Folk Medicinal Soil in China

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Abstract

Objective: To discover novel antimicrobial medicinal resource and reveal the anti-infection reasons of a folk medicinal soil.

Methods: The antimicrobial activities of sixty-one actinomycete strains isolated from a folk medicinal soil sample were evaluated using agar well diffusion and broth microdilution methods, and then seven strains with broad-spectrum antimicrobial activities were identified using polyphasic taxonomy procedure.

Results: Thirteen strains presented antimicrobial activities, and seven strains showed broad-spectrum antimicrobial activities with the minimum inhibitory concentrations (MICs) of 0.256-4.096 mg/ml against four pathogenic microorganisms including methicillin-susceptible Staphylococcus aureus, methicillin-resistant S. aureus (MRSA) and Candida albicans. Moreover, strain ZZ035 also showed antimicrobial activities with the MICs of 0.256-1.024 mg/ml against Escherichia coli. The identifications of these seven strains showed that all of them belonged to the genus Streptomyces. Furthermore, strain ZZ016 presented a high potential to be a new species.

Conclusion: These streptomyces strains were deduced to be mainly responsible for the anti-infection effect of the folk medicinal soil, and further research on their secondary metabolites may lead to the discovery of remarkable and broad-spectrum antibiotics, especially that of anti-MRSA ones.

Key Words:
Antimicrobial activity; Identification; Actinomycetes; Anti-MRSA; Streptomyces

Introduction

Around the reservoir for domestic water in Chinese rural areas, a folk medicinal soil is used to prevent infection and accelerate cure by being spread around the wound after dog bite, and lies in a dark and moist environment contaminated with edible oil, table salt and vinegar. To reveal the anti-infection reasons of the soil and discover actinomycetes strains with remarkable antimicrobial activities, a soil sample was collected in Xianjing Countryside in Zhuzhou County, China. After comparing the practical effects on wounds treated by a fresh soil sample and by a sterilized one, we inferred that the microorganism inhabited in the soil should be a key factor to prevent wound infection. As potential producers of bioactive metabolites [1,2], actinomycetes play an important role in the discovery of new antibiotics [3]. So, sixty-one actinomycete strains ZZ01 to ZZ061 were selectively isolated from 5 g of soil sample with improved Gauss medium, and further purified by yeast extract-malt extract agar (ISP 2) medium [4].

Various methods including bioactivity-directed, experimental, physicochemical and chemical strategies have been developing to discover new bioactive metabolites [5-7]. To discover actinomycete strains producing a series of secondary metabolites, chemical strategy was handily used to research on these sixty-one strains in our previous work [4]. By focusing on the structures of natural products, chemical strategy was more efficient to discover bioactive metabolites with special structure-types [8,9], however this will probably lead to the loss of some important bioactivity information. Therefore, the previous work was insufficient to explain the anti-infection reasons of folk medicinal soil, and some molecules with no ultraviolet-visible absorption were undetectable because of the limitation of technology we used. So, further research on these strains need to be made to broaden the scope of our previous work. As the soil is used to prevent infection, it is more necessary for us to adopt antimicrobial activity-directed approach to evaluate these actinomycete strains and legitimately explore the anti-infection reasons of the soil.

Using pathogenic microbe Staphylococcus aureus, Escherichia coli and Candida albicans as indicators, the antimicrobial activities of sixty-one strains were evaluated by agar well diffusion and broth microdilution methods. The results showed thirteen strains presented activities against methicillin-susceptible S. aureus (MSSA), E. coli and/or C. albicans, and seven strains had remarkable broad-spectrum antimicrobial activities. Considering the research and development of new anti-multidrug-resistant organisms (Anti-MDROs) drugs is particularly urgent [10,11], the anti-MDROs activities of these strains were further evaluated by using several methicillin-resistant S. aureus (MRSA) strains as indicators, and the results indicated that eight strains showed activities against MRSA. As seven strains showed...
remarkable broad-spectrum activities against MRSA and *C. albicans*, their identifications proceeded to clarify the anti-infection reasons of the soil and to establish a foundation of the successive researches on antimicrobial metabolites produced by these strains.

**Materials and methods**

**Actinomycete strains from a folk medicinal soil**

The isolation, purification and preservation of actinomycete strains can refer to our previous paper [4]. In a short, a soil sample was collected around a reservoir in Xianjing Countryside in Zhuzhou County, China (Geographic coordinates: 27°30’ N, 113°17’ E). Sixty-one actinomycete strains were selectively isolated from this soil with improved Gaus medium, and purified with ISP-2 medium. All these strains were stored in our lab at College of Bioscience and Bioengineering, Jiangxi Agricultural University, Nanchang, China.

**Pathogenic strains**

Two pathogenic strains *C. albicans* ATCC 10231 and *E. coli* S002 were friendly presented by Chinese Academy of Tropical Agricultural Sciences, Haikou, China. Two reference strains MRSA ATCC 33592, MSSA ATCC 25923 were purchased from American Type Culture Collection, and two clinical isolates of MRSA as MRSA 01 and MRSA 03 were obtained from Clinic Laboratory of Second Affiliated Hospital, Sun Yat-sen University, Guangzhou, China. Each strain inoculated on a special medium at appropriate temperature until the OD_{600} nm value was 0.60 before use.

**Antimicrobial activity of actinomycete strains**

According to the methods reported in our previous works [4], all actinomycete strains were cultured with 2A and soybean meal media respectively obtain 40 ml broth. After 2 ml of each broth was centrifuged to obtain supernatant as a test sample for antimicrobial assays, four times ethanol (v/v) was added into the remain broth of each strain. The suspension was ultrasonicated for 25 min at 40, and then was centrifuged at 12 000 r.p.m for 15 min. The supernatant was concentrated under vacuum to remove ethanol, and further dried by lyophilization to obtain an extract powder used as another test sample for antimicrobial assays.

Antimicrobial activities were determined by using agar well diffusion as described in our previous paper [4] with little modification. Each extract powder prepared in paragraph “2.3.1.” was dissolved in DMSO-H_{2}O (v/v, 3:2) to obtain test samples with the concentration of 8.192 mg/ml, while the supernatant of each strain was directly used for testing. Diluted inoculums (0.1 ml, 107 CFU/ml) of three strains *C. albicans* ATCC 10231, MSSA ATCC 25923 and *E. coli* S002 were respectively spread on YPD agar, Muller-Hinton agar (Qingdao Hope Bio-Technology Co., Ltd., China) and LB agar plates. After the agar surface dried, each well with a diameter of 8 mm was punched, and into which 100 μl test samples were added. Amphotericin B (128 mg/ml), oxacillin sodium (128 mg/ml) and kalanycin (128 mg/ml) were respectively used as positive controls for *C. albicans*, *S. aureus* and *E. coli*. DMSO-H_{2}O (v/v, 3:2) was used as solvent blank. The plates spread on *S. aureus* or *E. coli* were incubated at 37 °C for 24 h, while those spread on *C. albicans* were incubated at 28 °C for 24 h. With three replicates, the diameter of inhibition zone was measured, and described as (mean ± s) in mm. Using pathogenic strain MRSA ATCC 33592, the anti-MRSA activities of those samples with anti *S. aureus* activities were further evaluated by the same methods, and 32 mg/ml linezolid was used as positive control.

The MICs of those extract powders with antimicrobial activities were determined by broth microdilution method reported in our previous paper [4] with a minor modification. Briefly, starting with initial concentration of 8.192 mg/ml for each extract powder (100 μl) were mixed with 100 μl Muller-Hinton broth, and then the twofold dilution was followed. One hundred microliter diluted inoculums (1.0 × 107 CFU/ml) were added to each well, and the plates were incubated at 35 °C for 24 h. When the microbial growth in the well of solvent blank was sufficient, the MIC of each sample was determined as the lowest concentration visibly inhibited the microbial growth. If necessary 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) stain method was used to clearly observe the results.

**Identification of actinomycete strains**

The morphological characteristics of these actinomycete strains were determined by macroscopic and microscopic methods after incubation of targeted strains at 28 °C on Yeast Extract-Malt Extract Agar (ISP-2) medium. Their cultural characteristics were macroscopically described with, the e.g. growth, shape and color of aerial mycelium and substrate mycelium, soluble pigment etc. Through observing the mycelial structures under the microscope, their microscopic characteristics were determined with coverslip culture technique [11]. Compared their morphologies with those of actinomycete morphologies provided in Bergey’s Manual, these actinomycete strains were presumptively identified [12].

Various tests were carried out to determine the physiological and biochemical characteristics of targeted strains. Base medium consisting of (NH_{4})_{2}SO_{4} (g), KH_{2}PO_{4} (g), K_{2}HPO_{4} (g), MgSO_{4}·7H_{2}O (1.0 g), trace salts solution (1 ml) and agar powder (20 g) per one liter of ionized water was used for sugar utilization tests. Trace salts solution was prepared by dissolving CuSO_{4}·5H_{2}O (0.64 g), FeSO_{4}·7H_{2}O (0.11 g), MnCl_{2}·4H_{2}O (0.79 g) and ZnSO_{4}·7H_{2}O (0.15 g) in 100 ml ionized water. Medium ISP-2 was used for temperature tolerance, NaCl tolerance and pH tolerance experiments.

All targeted strains were incubated in ISP-2 liquid medium at 28°C for 7 days, and the suspensions were respectively centrifuged to obtain mycelia for chemical characteristic analyses. The cell-wall compositions were determined using thin layer chromatography (TLC) with microcrystalline cellulose used as matrix [13]. 2,6-diaminopimelic acid (DAP) isomers LL-DAP and meso-DAP were used as controls for cell wall amino acid analyses, and rhamnose, mannose, xylose, arabinose, glucose and galactose were used as controls for cell wall sugar analyses. The cell membrane phospholipids were analyzed using dual direction TLC [14].

The genomic DNA used for the PCR was extracted from the single colonies grown on ISP-2 medium at 28 for 3 days according to the protocol of SK8255 Kit (Sangon Biotech (Shanghai) Co., Ltd., China). Using Applied Biosystems® 2720 Thermal Cycler, the PCR amplifications with a primer pair 27F-1492R [15] carried out. Each 25 μl PCR reaction mixture contained 0.5 μl 20-50 ng/μl bacteria genomic DNA, 2.5 μl 10× PCR buffer with 20 mM MgCl_{2}, 1.0 μl 10 mM dNTPs, 0.2 unit Taq polymerase (MBI), 0.5 μl 10 μM for each primer. The cycling parameters were described as follows: Initial denaturation at 94 for 4 min; 30 cycles at 94 for 5 s, annealing at 54 for 45 s and primer extension at 72 °C for 1 min; followed by a final extension at 72 °C for 10 min. The PCR amplification products were resolved using
electrophoresis in 1.0% agarose gel, and purified according to the protocol of SK8131 Kit (Sangon Biotech (Shanghai) Co., Ltd, China). The 16S rRNA gene sequences were determined using the automatic sequencer Applied Biosystems® 3730XL DNA Analyzer. The 16S rRNA gene sequences were aligned against sequences of reference strains using the BLAST program (http://www.ncbi.nlm.nih.gov/). All the selected DNA multiple sequences were matched by means of software package Clustal X [16], evolution distances were calculated using Kimura2-Parameter model of MEGA version 4.0 [17]. The phylogenetic tree was constructed using the neighbor-joining algorithms [18]. Based on 1000 replicates, the confidence coefficient of the phylogenetic tree was evaluated using bootstrap analysis [19].

**Results and discussion**

**Antimicrobial activity of actinomycete strains**

| Strains | Medium | S. aureus | E. coli | C. albicans | MRSA ATCC 33592 |
|---------|--------|-----------|---------|-------------|-----------------|
|         |        | Extract   | Supernatant | Extract   | Supernatant | Extract   | Supernatant | Extract   | Supernatant |
| ZZ001   | 2A     | 12.7 ± 0.5 | 21.3 ± 1.6 | —          | —          | —        | —          | —        | —          |
|         | Soybean| —         | —         | —          | —          | —        | —          | —        | —          |
| ZZ003   | 2A     | 11.5 ± 0.3 | 12.5 ± 0.7 | —          | —          | —        | —          | —        | —          |
|         | Soybean| 10.3 ± 0.9 | 11.2 ± 0.5 | —          | —          | —        | —          | —        | —          |
| ZZ016   | 2A     | 23.2 ± 1.6 | 15.9 ± 1.1 | —          | —          | 20.8 ± 1.9 | 17.2 ± 1.3 | 24.5 ± 1.9 | 15.3 ± 1.2 |
|         | Soybean| 13.4 ± 1.0 | 12.8 ± 0.5 | —          | —          | 14.6 ± 1.2 | 15.3 ± 1.0 | 12.9 ± 1.0 | 12.2 ± 0.8 |
| ZZ018   | 2A     | 14.2 ± 0.9 | 19.3 ± 1.3 | —          | —          | 16.1 ± 1.0 | 14.4 ± 0.5 | 15.3 ± 1.4 | 20.1 ± 1.6 |
|         | Soybean| —         | —         | —          | —          | —        | —          | —        | —          |
| ZZ021   | 2A     | 20.4 ± 1.5 | 24.7 ± 1.5 | —          | —          | —        | —          | —        | —          |
|         | Soybean| —         | —         | —          | —          | —        | —          | —        | —          |
| ZZ024   | 2A     | 11.7 ± 0.5 | 16.2 ± 1.2 | —          | —          | —        | —          | —        | —          |
|         | Soybean| 12.1 ± 0.8 | 11.7 ± 0.5 | —          | —          | —        | —          | —        | —          |
| ZZ027   | 2A     | 32.2 ± 2.9 | 30.3 ± 2.6 | —          | —          | 22.6 ± 2.0 | 15.3 ± 1.3 | 30.8 ± 2.6 | 30.7 ± 3.0 |
|         | Soybean| 12.5 ± 0.7 | 13.2 ± 0.9 | —          | —          | 14.2 ± 1.1 | 13.9 ± 1.3 | 12.1 ± 0.5 | 12.7 ± 0.7 |
| ZZ031   | 2A     | 13.1 ± 1.1 | 17.8 ± 1.3 | —          | —          | —        | —          | —        | —          |
|         | Soybean| 11.2 ± 0.5 | 11.9 ± 0.8 | —          | —          | —        | —          | —        | —          |
| ZZ035   | 2A     | 31.6 ± 2.7 | 29.4 ± 2.2 | 23.5 ± 1.9 | 21.1 ± 1.5 | 28.3 ± 2.6 | 27.5 ± 2.3 | 32.5 ± 2.8 | 28.1 ± 2.6 |
|         | Soybean| 13.9 ± 1.0 | 12.3 ± 0.5 | —          | —          | 13.2 ± 1.0 | 12.1 ± 0.7 | 13.4 ± 0.7 | 12.5 ± 1.1 |
| ZZ036   | 2A     | 17.9 ± 1.3 | 22.5 ± 1.7 | —          | —          | 15.3 ± 1.0 | 17.9 ± 1.5 | 16.6 ± 1.5 | 21.9 ± 1.8 |
|         | Soybean| —         | —         | —          | —          | —        | —          | —        | —          |
| ZZ043   | 2A     | 22.8 ± 1.8 | 27.4 ± 2.0 | —          | —          | 23.5 ± 1.9 | 22.1 ± 1.7 | 22.4 ± 1.7 | 28.1 ± 2.5 |
|         | Soybean| —         | —         | —          | —          | —        | —          | —        | —          |
| ZZ048   | 2A     | 13.6 ± 1.0 | 10.7 ± 0.6 | —          | —          | —        | —          | —        | —          |
|         | Soybean| 14.1 ± 0.7 | 12.6 ± 1.0 | —          | —          | —        | —          | —        | —          |
| ZZ057   | 2A     | 34.1 ± 3.5 | 32.9 ± 3.1 | —          | —          | 23.3 ± 2.5 | 21.5 ± 1.9 | 32.6 ± 3.4 | 30.3 ± 2.7 |
|         | Soybean| 28.5 ± 2.8 | 23.3 ± 2.4 | —          | —          | 18.5 ± 1.6 | 20.2 ± 1.6 | 27.5 ± 2.3 | 23.6 ± 1.9 |

*Actinomycete strains with no antimicrobial activities are not shown; b - Showed no inhibition zone.*

**Table 1:** Antimicrobial activities of the samples obtained from actinomycete strains (mean ± s, n = 3).
The antimicrobial assays showed thirteen actinomycete strains had antimicrobial activities (Table 1), and the percentage of actinomycete strains with antimicrobial activity was 21.3%. More importantly, seven of them presented broad-spectrum antimicrobial activities against S. aureus and C. albicans, and strain ZZ035 showed more broad-spectrum activities against all pathogenic microorganisms tested. As the pathogenic microorganisms including Gram-positive and Gram-negative germ (S. aureus and E. coli) or/and pathogenic fungus (C. albicans) inhibited by these actinomycete strains, these actinomycete strains should be responsible for the anti-infection effect after using this soil around the wound. Although chemical screening can lead to the discoveries of actinomycete strains against pathogenic microorganism, the activity-directed strategies show more efficient to discover antimicrobial actinomycete strains and to clarify the anti-infection reasons of the folk medicinal soil comparing the chemical screening of sixty-one actinomycete strains [4]. Considering that clinic drugs with anti-MDROs drugs are more and more rare [10,20], the anti-MDROs activities of thirteen strains with antimicrobial activities were further evaluated by using MRSA strains as indicators. Eight strains presented anti-MRSA activities, while other five strains inhibited MSSA showed no anti-MRSA activities. Furthermore, many actinomycete strains with antimicrobial activity against S. aureus (occupied 21.3%) and C. albicans (occupied 11.5%) were isolated from the soil sample, while just one strain presented anti E. coli activity. This also indicated that it is more difficult to discover that natural products inhibited Gram-negative microbes than Gram-positive ones [21,22].

To evaluate the antimicrobial potency of thirteen actinomycete strains, the MICs of the extract powders from their broths cultured in the soil sample, while just one strain presented anti E. coli activity. This also indicated that it is more difficult to discover that natural products inhibited Gram-negative microbes than Gram-positive ones [21,22].

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Table 2: Minimum inhibitory concentration (MICs) against pathogenic microorganism of the extract powders from actinomycete strains with antimicrobial activities.

| Strain   | MICs against pathogenic microorganism (mg/ml) | S. aureus | E. coli | C. albicans | MRSA ATCC 33592 | MRSA 01 | MRSA 03 |
|----------|---------------------------------------------|-----------|---------|-------------|-----------------|---------|---------|
| ZZ001    |                                             | 0.096     | —       | —           | —               | —       | —       |
| ZZ003    |                                             | 0.096     | —       | —           | —               | —       | —       |
| ZZ016    |                                             | 1.024     | 1.024   | 1.024       | 0.512           | —       | —       |
| ZZ018    |                                             | 0.096     | 2.048   | 4.096       | 4.096           | 4.096   | 4.096   |
| ZZ021    |                                             | 2.048     | —       | 2.048       | 2.048           | 2.048   | 2.048   |
| ZZ024    |                                             | 0.096     | —       | —           | —               | —       | —       |
| ZZ027    |                                             | 0.512     | 1.02    | 0.256       | 0.512           | 0.512   | 0.256   |
| ZZ031    |                                             | 0.512     | 1.02    | 0.256       | 0.512           | 0.512   | 0.256   |
| ZZ035    |                                             | 2.048     | —       | 2.048       | 2.048           | 2.048   | 2.048   |
| ZZ043    |                                             | 1.024     | 0.512   | 1.024       | 0.512           | 0.512   | 0.512   |
| ZZ048    |                                             | 4.096     | —       | —           | —               | —       | —       |
| ZZ057    |                                             | 0.256     | 0.512   | —           | 0.256           | 0.256   | 0.256   |
| Oxacillin sodium |                     | 0.50       | —       | —           | —               | —       | —       |

Table 3: Cultural characteristics of seven targeted strains on ISP2 medium for 14 days.

| Strain   | Growth | Substrate mycelium | Soluble pigment | Substrate mycelium | Soluble pigment |
|----------|--------|-------------------|----------------|-------------------|----------------|
| ZZ016    | Good, wrinkled and radial, compatible | White with a little fuchsia | Pale brown | None |
| ZZ018    | Good, wrinkled and radial, uncompatible | White with a little fuchsia | Pale brown | None |
| ZZ027    | Moderate, point-like and convex colonies | White | yellowish brown | None |
| ZZ035    | Good, coral-like | White with a little grey red | yellowish brown | None |
| ZZ036    | Good, wrinkled and radial | Purple | Purple | |
| ZZ043    | Good, wrinkled and radial | White | yellowish brown | None |
| ZZ057    | Good, coral-like | White with a grey red | yellowish brown | None |

Many researches showed that the biofilm formation is an immune evasion strategy and a reason for MRSA to be resistant to various drugs [23-25]. Simultaneously, S. aureus together with C. albicans can form poly-microbial bio-film for antimicrobial resistance [26], and Candida albicans has the ability to induce the resistance of S. aureus to some drugs during polymicrobial biofilm formation [27]. Therefore, seven actinomycete strains with broad-spectrum activities against MRSA and C. albicans showed a great potential to discover efficient anti-MRSA metabolites.
purple soluble pigment while other six strains had no ability to produce soluble pigment. It is worth noting that the cultural characteristics of strains ZZ016 and ZZ035 are similar to those of strains ZZ018 and ZZ057, respectively. The obvious difference between the cultural characteristics of ZZ016 and ZZ018 was that the colonies of strain ZZ016 showed harmonious growth while those of strain ZZ018 showed antagonistic one (Figure 1). The cultural characteristics of ZZ035 and ZZ057 showed no obvious difference, while the mycelia of strain ZZ057 had diaphragms which were observed from its microscopic characteristic (Figure 2), but those of strain ZZ035. Furthermore, strains ZZ016 and ZZ018 also have diaphragms observed from Figure 2, while other three strains have no diaphragms.

Figure 1: The cultural characteristics of ZZ016 and ZZ018.

Figure 2: Microscopic characteristics of strains ZZ016 (a), ZZ018 (b), ZZ035 (c) and ZZ057 (d) – 400.

The physiological and biochemical characteristics of targeted strains were presented in Table 4. Sugar utilization tests indicated that all these strains grew well when glucose, maltose, sucrose or glycerol used as a sole carbon source. Good growth for most strains was observed between 25-35 °C, and the maximum temperature for the growths of these strains was at 45 °C. Except that strains ZZ035 and ZZ057 grew on medium ISP-2 contained no more than 3.0% NaCl, all of them could grow on that contained no more than 5.0% NaCl and even that strains ZZ016 and ZZ036 could grow on that contained 7.0% NaCl. It is distinctly different from other strains that 1.0% to 2.0% NaCl could promote the growth of strain ZZ027. The pH value for suitable growth was 4.0 to 7.0 for strains ZZ035 and ZZ043 while that was 4.0 to 8.0 for other five strains. The higher NaCl tolerance and lower pH tolerance of these actinomycete strains should be relative to the high salt-acid survival environment under which the soil exposed to salt, vinegar, edible oils and fats in the farmhouses.
Furthermore, the high bootstrap value (99% to 100%) and 16S rRNA gene sequence similarities (98.7% to 99.2%) indicated strains ZZ035 strain ZZ016 had a high potency to be a new species. The chemical characteristics, together with their individual morphological, physiological and biochemical ones, indicated that seven targeted actinomycete strains should likely belong to the genus Streptomyces [12-14].

Analysis of the 16S rRNA gene sequence exhibited that all seven strains presented a very high level (96.9% to 99.5%) of 16S rDNA sequence similarity with their closest streptomyces species respectively deposited in GenBank. The closest relationship of seven targeted strains to members of the genus Streptomyces, together with their morphological, physiological and biochemical characteristics indicated these strains belonged to the genus Streptomyces. Considering their activities against MRSA and C. albicans, these seven streptomyces strains isolated from the folk medicinal soil should be mainly responsible for the anti-infection activity of the soil, and deposited at NCBI GenBank with accession numbers KJ995740, KJ995742, HQ874465, KJ995743, KJ995741, KJ995744 and KJ995739 respectively for strains ZZ016, ZZ018, ZZ027, ZZ035, ZZ036, ZZ043 and ZZ057.

Table 4: Physiological and biochemical characteristics of targeted strains.

|      | a- | +  | ++ | +++ | +    | ++   | +    | +    |
|------|----|----|----|-----|------|------|------|------|
| 7.0  |    |    |    |     |      |      |      |      |
| 8.0  |    |    |    |     |      |      |      |      |
| 9.0  |    |    |    |     |      |      |      |      |
| 10.0 |    |    |    |     |      |      |      |      |

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Figure 3: Neighbour-joining tree based on nearly complete 16S rRNA gene sequences.

The phylogenetic tree based on the neighbor-joining algorithms was shown in Figure 3. Strains ZZ016 grouped together with ZZ018 under a moderate bootstrap value of 71%, and these two strains together with strain ZZ057 formed a clade with Streptomyces albireticuli by a moderate bootstrap value (67%). While the 16S rRNA gene sequence similarity (96.9%) between strains ZZ016 and S. albireticuli showed strain ZZ016 had a high potency to be identified as a new species. Furthermore, the high bootstrap value (99% to 100%) and 16S rRNA gene sequence similarities (98.7% to 99.2%) indicated strains ZZ035 and ZZ043, ZZ027 and ZZ036 were likely subspecies of S. cinnamonomensis, S. cangkringensis and S. californicus, respectively.

Conclusions

Further researching on sixty-one actinomycetes strains isolated from a folk medicinal soil sample, thirteen strains presented obvious activities against S. aureus, E. coli and/or C. albicans. Seven of them showed broad-spectrum antimicrobial activities against MRSA and Candida albicans, and strain ZZ035 showed more broad-spectrum antimicrobial activities against all pathogenic microorganisms tested including Escherichia coli. Polyphasic taxonomy procedure showed that all of them were close to each other in taxonomy, and belonged to the genus Streptomyces. Furthermore, strain ZZ016 presented a high potential to be a new species. These streptomycete strains were deduced to be mainly responsible for the anti-infection effect of the folk medicinal soil, and further research on their secondary metabolites may lead to the discovery of remarkable and broad-spectrum antibiotics, especially for anti-MRSA ones.

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