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Research Article

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Posted Date: March 22nd, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-313547/v1>

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Antimicrobial compounds were isolated from the secondary metabolites of endophytic *Gordonia* in the intestinal tract of *Periplaneta americana*

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Abstract

Background: As we all know, bacterial and fungal infections have become one of the threats to human health. Microbial secondary metabolites are one of the main sources of bioactive natural products. It is estimated that around 60% of all foregone antibiotics are derived from secondary metabolites produced by filamentous actinomycete bacteria. *Gordonia* spp. are members of the actinomycete family, their contribution to the environment improvement and environmental protection by their biological degradation ability, but there are few studies on their antimicrobial activity of their secondary metabolites. Our team isolated a *Gordonia* strain WA 4-31 with anti-*Candida albicans* activity from the intestinal tract of *Periplaneta americana* in the early stage.

Results: In this study, we firstly identified the strain WA 4-31 by the morphological characteristics and the phylogenetic analyses, and found that it homologous to a strain of *Gordonia* from the Indian desert (EU333873) by 100%. Then four compounds, Actinomycin D (1), Actinomycin X₂ (2), Mojavensin A (3) and cyclic (leucine-leucine) dipeptide (4) were purified from the EtOH extract of the fermented broth of the strain. The compounds 1-4 had activities against *Candida albicans*, *Aspergillus niger*,

Aspergillus fumigatus and *Trichophyton rubrum*. They also had activities against MRSA, *S.aureus*, *K.pneumoniae* and *E.coli* in different degree. The minimum inhibitory concentration of Actinomycin D and Actinomycin X₂ on MASA was 0.25 µg/mL. Interestingly, we found that when Mojavensin A was mixed with compound 4 ratio of 1:1, the solution of the compounds was better than the single on anti-*Candida albicans*. Besides, compounds 1-3 had varying degrees of cytotoxicity on CNE-2 cells and HepG-2 cells.

Conclusions: The present study firstly reported the antimicrobial compounds isolated from *Gordonia*. These indicated that rare actinomycetes from the intestinal tract of *Periplaneta americana* possessed a potential as a source of active secondary metabolites.

Keywords: Endophytic *Gordonia* ; Secondary metabolites; Antimicrobial activity; Cytotoxicity; *Periplaneta americana*;

Introduction

Microbial secondary metabolites are one of the main sources of bioactive natural products and are also one of the main sources of drugs. It is estimated that around 60% of all foregone antibiotics are derived from secondary metabolites produced by filamentous actinomycete bacteria, especially *Streptomyces* species. But over time, the continual rediscovery of known compounds from secondary metabolites of *Actinomyces* has resulted. Consequently, special extreme environment and growing conditions for bacteria raised much concerns in recent years[1], such as deep-sea environment, with characteristic features of high salinity, high pressure, low temperature and low nutrition [2, 3]; cold polar regions[4]; deserts[5, 6] or endogenous environment in insects and plants[7, 8].

Endophytic microorganisms of insects and plants have attracted much attention in recent years. They play important role of acting as reservoirs of novel bioactive secondary metabolites that serve as a potential candidate for antimicrobial, anti-insect, anticancer and many more properties [9]. In order to cope with the emergence of drug resistance, scientific efforts have been aimed at the bioprospecting of microorganisms' secondary metabolites, with special emphasis on the search for antimicrobial natural products derived from endophytes[10-12]. Silva et al. isolated and identified endophytic fungal communities from the roots and seeds of *P. cupana* genotypes susceptible and tolerant to anthracnose that grow in two sites of the Brazilian Amazonia forest. They also identified eight fungal species not previously reported as endophytes; some fungal species capable of inhibiting pathogen growth; and the production of antibiotics and compounds with bacteriostatic activity against *Pseudomonas aeruginosa* in both susceptible and multiresistant host strains[13].

Besides, in the existing research reports of insect endophytes, most of them focus on pest control, biological agents, ecological protection and ecological diversity, and the study on antibacterial activity of their secondary metabolites is less[14-17]. Our previous study showed that the endophytic strains in the intestinal tract of *Periplaneta americana* had various antibacterial activities against pathogenic bacteria. Fang et al. found one actinomycete strain WA23-4-4 isolated from the intestinal tract of *Periplaneta americana* that exhibited broad spectrum antifungal activity. 3-Acetyl benzoyl amide was isolated by ethyl acetate extraction of WA23-4-4 fermentation broth, and the compound showed significant activity against *Candida albicans* and *Aspergillus niger*[18]. Chen et al. also found one strain WA5-2-37 isolated from the intestinal tract of *Periplaneta americana*, exhibited great activity against MRSA[19]. The strain produced actinomycin X₂ and collismycin A had strong inhibition for MRSA with minimum inhibitory concentration (MIC) values of 0.25 and 8 µg/mL.

Rare actinomycetes have been paid more attention as the hope of discovering new antibiotics. At present, some antibiotics that have been used in clinic, such as gentamicin, erythromycin, vancomycin and rifampicin, are all from rare actinomycetes[20]. Rare actinomycete *Gordonia* are usually used for the environment improvement and environmental protection by its biological degradation ability, but there are few studies on its antimicrobial activity[21]. Our team isolated a *Gordonia* strain WA 4-31 with anti-*Candida albicans* activity from the intestinal tract of *Periplaneta americana*. In this study, four compounds were isolated and purified from the ethyl acetate extract of fermentation broth of the *Gordonia* strain WA 4-31 with antimicrobial activity, especially compounds 1-3 Actinomycin D (1), Actinomycin X₂ (2), Mojavensin A (3) showed better anti-fungal and anti-cancer activities. The study firstly reported the antimicrobial compounds isolated from *Gordonia* from the intestinal tract of *Periplaneta americana*. These indicated that *Gordonia* rare actinomycetes possessed a great potential as a source of active secondary metabolites.

Results

Identification of the strain WA 4-31

The colonies of strain WA 4-31 on Gauze's medium were milky yellow. The surface of the colony was smooth and moist, and its edge was irregularly convex (Fig. 1 (A)). The strain WA 4-31 was observed to be short rod-shaped and unbranched under electron microscope (Fig. 1 (B) (C)). The sequence of this strain WA 4-31 was submitted to GenBank of NCBI, and the accession number was MH613773. The strain WA 4-31 of the 16S rRNA gene sequence was compared by BLAST and homologous to a strain of *Gordonia* from the Indian desert (EU333873) by 100% (Fig. 1 (E)).

Isolation of Secondary Metabolites

The crude ethyl acetate extract (about 13.4 g) was firstly separated by silica gel column chromatography eluting with a gradient system of $\text{CHCl}_3:\text{CH}_3\text{COOC}_2\text{H}_5$ and $\text{CH}_3\text{COOC}_2\text{H}_5:\text{CH}_3\text{OH}$ to yield 20 fractions. Six fractions showed antibacterial activity against *Candida albicans* ATCC 10231 by Oxford Cup method, especially the 10th fraction showed stronger antibacterial activity, the diameters of inhibition zones of which was 36.0 ± 2.0 mm, while the others did not show activity (Fig.2 , Table 1). These fractions with anti-*Candida albicans* activity were repeatedly purified by silica gel column, ODS column, Sephadex LH-20 column, and HPLC to offer compound 1 (7.7 mg), compound 2 (24.2mg), compound 3 (34.1 mg) and compound 4 (35.9 mg).

Identification of Compounds

Compound 1, orange powder; HR-ESI-MS m/z 1255.46 $[\text{M}+\text{H}]^+$, m/z 1277.39 $[\text{M}+\text{Na}]^+$. Compound 2, orange powder; HR-ESI-MS m/z 1269.42 $[\text{M}+\text{H}]^+$, m/z 1291.44 $[\text{M}+\text{Na}]^+$. Compound 3, white solid; HR-ESI-MS m/z 1086.51 $[\text{2M}+\text{H}]^+$, m/z 1107.43 $[\text{M}+\text{Na}]^+$. Compound 4, colorless needle-like solid; HR-ESI-MS m/z 227.15 $[\text{M}+\text{H}]^+$. The chemical structure of compounds 1-4 was identified based on the spectral information relating to ^1H NMR and ^{13}C NMR spectral data (See Supplementary), which were respectively confirmed to be Actinomycin D (1), Actinomycin X₂ (2), Mojavensin A (3) and cyclic (leucine-leucine) dipeptide (4) by comparing the microspectrum, SciFinder and related references(Fig. 3).

Antibacterial activity of Compounds

The MIC experiment found that compounds 1-4 have broad spectrum antibacterial activity (Table 2), especially compound 3 Mojavensin A had the best effect on the four kinds of the tested fungi: *Candida albicans*, *Trichophyton rubrum*, *Aspergillus fumigatus* and *Aspergillus niger*. Compound 4 (leucine) -Leucine) dipeptide has the strongest inhibitory effect on *E. coli*, at 32 $\mu\text{g/mL}$. In addition,

compounds 1 Actinomycin D and compounds 2 Actinomycin X₂ have the best effects against MRSA (ATCC 43300), with an inhibitory effect of 0.25 µg/mL.

Interestingly, Mojavensin A and cyclic (leucine-leucine) dipeptide, which were separated from the tenth fraction with the strong activity against *Candida albicans* (Fig.4), mixed with different proportions had stronger activity against *Candida albicans* than every single compound. When the ratio was 1:1, the activity against *Candida albicans* was the strongest, but it still did not reach the strength of the tenth fraction (Table 3).

Cytotoxicity of Compounds

Cytotoxicity of Compounds 1-3 Actinomycin D, Actinomycin X₂ and Mojavensin A for HepG-2 and CNE-2 cells was performed by MTT at different time. Results show that compounds 1-3 could inhibited cell proliferation in a time-dependent manner (Fig.5). Meanwhile the inhibitory effect of them for HepG-2 had the concentration correlation. The antiproliferative effect of Actinomycin D and Actinomycin X₂ was more effectively than Mojavensin A at the Concentration of less than 50 µg/ml. According to the inhibition rate of different concentrations, the half-maximal inhibitory concentration (IC₅₀) of the compounds on HepG-2 and CNE-2 cells were calculated using the probability distribution of SPSS13.0 (Table 4).

Discussion

The growing resistance of microorganisms towards antibiotics has become a serious problem around the world. Therapeutics with novel chemical scaffolds and/or mechanisms of action are urgently needed to combat infections caused by multidrug resistant pathogens, including bacteria, fungi and viruses. Development of novel antimicrobial agents still highly depends on the new natural products. At present, most antimicrobial drugs used in medicine are of natural origin. Among the natural

producers of bioactive substances, Actinobacteria continue to be an important source of novel secondary metabolites for drug application[22]. It was estimated that from the first report of streptothricin in 1942 and streptomycin a year later, the order Actinomycetales had yielded about 3000 known antibiotics[23]. However, with the known antibiotics are discovered repeatedly, there are fewer new antibiotics found from *Streptomyces*. Rare actinomycetes have been paid more attention as the hope of discovering new antibiotics. 167 new bioactive compounds produced by 58 different rare actinomycete species representing 24 genera. A total of 97 new species, representing 9 novel genera and belonging to 27 families of marine rare actinomycetes had been reported from mid-2013 to 2017[24]. About 20 years ago, rare actinomycete *Gordonia* was described as a genus, and there are 39 different species have been identified so far[25]. Some *Gordonia* species cause a broad spectrum of diseases in healthy and immunocompromised individuals. Besides, these bacteria can produce useful secondary metabolites that may be used in various industries[26].

In this study, we validated the endogenous *Gordonia* isolated from the intestinal tract of *Periplaneta americana* by sequence and phylogenetic tree construction and we named this *Gordonia* strain WA 4-31. Phylogenetic data showed that the strain WA 4-31 was 100% homologous to *Gordonia* from the Indian desert. Colony conformed to the biomarker of *Gordonia* by colony phenotype. Previous studies have shown that the endogenous environment of insects is unique and the natural products from the endogenous environment of insects may be different from the common environment in the land. Therefore, it is speculated that insect endophytes may produce precious and interesting natural compounds[27-29]. Insects are a highly diverse group, exploit a wide range of habitats, and harbor bacterial symbionts of largely unknown diversity. Insect-associated bacterial symbionts are underexplored but promising sources of bioactive compounds[30]. Lee SR et al. reported the discovery

of three new cyclic tripeptides: natalenamides A-C[31]. These compounds were identified from the culture broth of the fungus-growing termite-associated *Actinomadura* sp. RB99. Li TX et al. reported two new compounds, versicolones A and B and three known pyrone derivatives were isolated from the insect-associated fungus *Aspergillus versicolor*[32]. At the present study, four antimicrobial compounds were isolated from the secondary metabolite of the strain WA4-31. Although these compounds, Actinomycin D, Actinomycin X₂ and Mojavensin A were reported, which found in *Gordonia* is the first time. There was no report that they were found in secondary metabolites at the same time so far.

In our study, Actinomycin D and Actinomycin X₂ showed extremely strong activity on MRSA, reached 0.25 µg/mL respectively, which was consistent with the existing reports[33-34]. These two Actinomycins showed general resistance to the remaining tested pathogens, which was different, compared with some reports. Xiong et al. found that Actinomycin X₂ had strong antibacterial activity against several Gram-positive and Gram-negative bacteria examined (*Staphylococcus aureus*, *Pseudomonas solanacearum*, *Escherichia coli*, etc.) and was especially effective against *S. aureus*, with MIC₅₀, 0.002 µg/ml; MIC₉₀, 0.017 µg/mL[35]. Actinomycin D from the study by Zhang et al. were evaluated for their activity against the growth of methicillin-resistant *S. aureus*, *E. coli*, and *C. albicans* using the micro broth dilution method and the results showed that Actinomycin D significantly inhibited the growth of both bacteria and fungi with MIC values of 0.08 to 9.96 µM[36]. Among compounds, Mojavensin A had the best inhibitory activity against *Aspergillus niger*, *Trichophyton rubrum* and *Aspergillus fumigatus*, reaching 32 µg/mL, 16 µg/mL and 64 µg/mL. The inhibitory effect of compound 3 Mojavensin A on *Escherichia coli* was 32 µg/mL. Mojavensin A was found in 2012 for the first time[37]. In another report said mojavensins displayed moderate antagonism and dose-dependent activity against several formae speciales of *Fusarium oxysporum* and presented surface

tension activities. These properties demonstrated that these lipopeptides may be useful as biological control agent to fungal plant pathogens[38]. These reports provided good evidence for our study on antibacterial activity in this article. The antibacterial activity of compound 1-3 against *Candida albicans* ranged from 32-128 µg/mL.

In addition, we found a more interesting phenomenon. In the process of separating and purifying the secondary metabolites of strain WA 4-31, it was found that one of the fractions showed strong activity against *Candida albicans* (Fig. 2; Table 1). Compound 3 Mojavensin A and compound 4 cyclic (leucine-leucine) dipeptide were obtained by further separation and purification from this fraction. The anti-*Candida albicans* activity of these two compounds was determined, and the activity of single compound was not outstanding. Then we mixed these two compounds according to different proportions, and it was found that the activity was obviously improved, but it still did not reach the strength of the fraction (Fig.4; Table 3). Antimicrobial resistance threatens a resurgence of life-threatening bacterial infections and the potential demise of many aspects of modern medicine. In order to delay the problem of antimicrobial resistance, people have extensively studied the synergistic effect of drugs in recent years. Combinations of antibiotics and of antibiotics with non-antibiotic activity-enhancing compounds offer a productive strategy to address the widespread emergence of antibiotic-resistant strains[39-40]. As there are still other complex bioactive components in the fractions that have not been separated, the synergistic effect and its mechanism among the specific compounds with strong anti-*Candida albicans* activity have not yet been determined, which needs further study. The inhibitory activities of all compounds on different bacteria and fungi were not consistent, so it was speculated that their coexistence was complementary for the host. Perhaps the host used them to fight against different pathogenic microorganisms to adapt to various living environments.

At the same time, it also showed that the diversity and richness of secondary metabolites were conducive to the adaptation of microorganisms to their living environment for microorganisms themselves, which is of great significance[41].

In the cytotoxicity test, the cytotoxic effects of compounds 1-3 on different various of cells were different, and most of them were time-dependent or concentration-dependent. In the previous studies, Actinomycin D had a good anti-cancer effect on specific cancer cells, but its toxic and side effects limit its wide use[42, 43]. In this study, Actinomycin D could inhibit CNE-2 cells proliferation within 24 h, reaching its peak at 72 h. The inhibition of Actinomycin X₂ on CNE-2 cells growth was not concentration-dependent, but time-dependent. Two kinds of Actinomycins inhibited the growth of HepG-2 cells in a time-dependent manner. At 48-72 h, Actinomycin X₂ with higher concentration had obvious inhibitory effect on the growth of HepG-2 cells. We know that the structures of Actinomycin D and Actinomycin X₂ are similar, but they showed inconsistent anticancer effects in this study. Actinomycin D has worked well in the treatment of Wilms tumors and neuroblastoma, and low-dose agents have been clinically applied[44]. About Actinomycin X₂, it was reported that apoptosis of human prostate cancer cells could be induced by marine Actinomycin X₂ but was not widely used in clinic[45]. At present, there is no research report about the anticancer effects of Actinomycin D and Actinomycin X₂ on these two cancer cells. Mojavensin A can promoted the growth of HepG-2 cells within 24 h, with a certain concentration correlation. However, after 48 h, Mojavensin A began to inhibit the growth of HepG-2 cells. The inhibitory effect of Mojavensin A on the growth of CNE-2 cells had obvious time dependence. There were very few reports about anti-cancer research of Mojavensin A, only one report revealed cytotoxic activities of Mojavensin A against the human leukemia (HL-60) cells line, with IC₅₀ of 100 μ M[37]. Through this work, we hope to provide some research basis for the

anti-cancer activities of these compounds. The mechanism of inhibiting proliferation of cancer cells are still unclear and need further study.

Through our work, we identified a rare actinomycete strain WA 4-31 from the intestinal tract of *Periplaneta americana* as *Gordonia*. Four compounds with antibacterial activity, Actinomycin D, Actinomycin X₂ and Mojavensin A, were isolated and purified from the secondary metabolites of the strain WA 4-31. We determined their antibacterial activity and inhibitory effect on CNE-2 cells and HepG-2 cells. In the experiment of determining the anti-*Candida albicans* activity, we found that Mojavensin A and cyclic (leucine-leucine) dipeptide had a synergistic effect after being mixed in different proportions, which could enhance the anti-*Candida albicans* activity. This phenomenon was a good enlightenment for us, maybe we can continue our in-depth study in the follow-up study on antibacterial activity.

Material and Methods

Microorganisms, Cell lines

The *Gordonia* Strain WA 4-31(MH613773) was isolated from the intestinal tract of *Periplaneta americana* and saved by Guangdong Key Laboratory of Pharmaceutical Bioactive Substances. The tested strain including four kinds of pathogenic fungi: *Candida albicans* ATCC 10231, *Trichophyton rubrum* ATCC 60836, *Aspergillus niger* ATCC 16404, *Aspergillus fumigatus* ATCC 96918, and four kinds of pathogenic bacteria: methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC 43300, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and *Pneumobacillus* ATCC 13883, were obtained from Guangdong Institute of Microbiology. CNE-2 Human nasopharyngeal carcinoma cell line, HepG2 human hepatocellular carcinoma cells were purchased from Experimental Animal Center of Sun Yat-sen University and Shanghai Cell Biology Institutes respectively.

Media and Chemicals

Microbiological media were purchased from Guangdong Huankai Microbial Co. The genomic DNA isolation kit was purchased from TIANGEN Biotech. High-performance liquid chromatography (HPLC) grade methanol was purchased from ThermoFisher. Silica gel used for extraction and column chromatography were obtained from Qingdao Ocean Chemical Co. Sephadex LH-20 dextran gel, CHP20P MCI and DIASOGEL ODS were purchased from Mitsubishi Chemical Co. of Japan. Solvents used for extraction and column were of analytical grade which were obtained from Guangdong Guanghua Sci-Tech Co.

Identification of *Gordonia* Strain WA 4-31

The *Gordonia* strain WA 4-3 was inoculated on Gauze's No. 1 medium and incubate at 28°C for 3 days. Three days later, the single colony was selected and cultivated in the ISP-1 seed medium at 28°C for 2 days. The morphology and surface characteristics of the bacterial colonies were examined using a scanning electron microscope (Hitachi S-3400N, Japan). Whole genomic DNA of the strain was extracted according to the manufacturer's instructions of the genomic DNA isolation kit. The 16S rDNA gene was then amplified by PCR, using universal bacterial primer (27F: 5'-AGA GTT TGA TCC TGG CTC AG-3' and 1492R: 5'-TAC GGC TAC CTT GTT ACGACT T-3'). PCR products were visualized by electrophoresis and was sequenced in BGI. The resulting sequence was compared to all sequences available in GenBank using the BLAST software from the National Center for Biotechnology Information (NCBI) website (<http://www.ncbi.nlm.nih.gov/>). The phylogenetic tree was constructed by MEGA.

Production and Isolation of Secondary Metabolites from Strain WA 4-31

The ISP 2 medium was used as the production culture. A 9 mL portion of the seed culture was

transferred into a 500-mL Erlenmeyer flask containing 300 mL of the ISP 2 medium and incubated on rotary shakers (160 rpm) at 28°C for 21 days. The total 63L of culture broth was extracted 3 times with an equivalent volume of ethyl acetate. After extraction and evaporation, 13.4g crude ethyl acetate extract was obtained. The crude ethyl acetate of the strain WA 4-31 was separated to given 20 major fractions (1–20) using silica gel. The crude extract and every fraction were tested for antimicrobial activity against *Candida albicans* ATCC 10231 by Oxford Cup method[46]. Finally, the active fractions were further purified by ODS column chromatography, Sephadex LH-20 column, and high-performance liquid chromatography (HPLC) to offer compounds.

Spectroscopic Analysis

The molecular weight of the purified compounds was determined using mass spectrometry and their structure was characterized by ¹H NMR and ¹³C NMR. The compounds were fully dissolved in methanol with a concentration of about 1 mg/L, filtered with a 0.22 µm filter membrane, and then mass spectra was obtained within the range of m/z100-1500 by TSQ Endura™ Triple-quadrupole mass spectrometer (Thermo Scientific, USA). Selecting appropriate 600µL of deuterated reagent to dissolve 10.0 mg of the compounds, the proton and carbon nuclear magnetic resonance (NMR) spectra were recorded at 600 MHz using a Bruker AVANCE III 600M spectrometer (Bruker, Germany).

Determination of minimum inhibitory concentration (MIC)

Minimum inhibitory concentration (MIC) measurements of compounds against the tested strain were performed by a microdilution method. The compounds were dissolved in DMSO and then diluted by the twofold dilution method. One hundred microliters of bacterial suspension or fungi suspension (10⁶ CFU/mL) were added to the 96-well plates. Then one hundred microliters of compounds were added to the 96-well plates respectively. The final concentrations of each sample in the wells were

512, 256, 128, 64, 32, 16, 8, 4, 2, 1, 0.5 and 0.25 µg/mL. Amphotericin B, Vancomycin, Ampicillin and Ciprofloxacin were used as positive drugs for fungi, MRSA, *Pneumococcus* and *Escherichia coli* respectively. The MIC value of the compounds was determined as the lowest concentration that completely inhibited bacterial growth after 48 h of incubation at 37°C, while the fungi was incubated at 28°C. All experiments were performed in triplicate.

Filter paper diffusion method

Antibacterial activity against *Candida albicans* was tested using the filter paper diffusion method. Sterile filter paper discs (6mm) were taken, soaked sufficiently in different samples for 5 min and set aside. Bacterial suspensions were dipped with sterile cotton, and uniformly applied in the culture media. Fully soaked filter paper was placed in the bacteria containing plates and cultured for 24 h in the 28°C constant temperature incubator. Amphotericin B (32 µg/mL) was used as positive control. The experiment was performed in triplicate and the mean of the diameter of the inhibition zones was calculated.

Cells viability assay

Cellular viability was evaluated with the MTT (Amresco, USA) assay. 5×10^3 cells suspended in 100 µl DMEM medium were added into a 96-well plate and treated with different concentrations of compounds for 24 h /48 h /72 h. After then, 10 µL of 0.5 mg/mL MTT reagent was added to each well for an additional 4 h. The purple formazan crystals were dissolved with 100 µL DMSO per well for further quantitative measurement at an absorbance of 570 nm using a microplate spectrophotometer. Experiments were independently repeated three times.

Abbreviations

MRSA: Methicillin-resistant *Staphylococcus aureus*; HPLC: High Performance Liquid Chromatography; NMR: Nuclear Magnetic Resonance; HR-ESI-MS: High-resolution electrospray ionization mass spectroscopy; EtOAc: Ethyl acetate; MEGA: Molecular Evolutionary Genetics Analysis; DMSO: Dimethyl Sulfoxide; CFU: Colony-forming Unit

Acknowledgment

The authors gratefully acknowledges Ph.D Xiongming Luo for analyzing the results of compounds.

Authors' contributions

XJ devised and supervised the experiments. MX, HL, TY and PG participated in the experiments. WL analyzed data. YM, MX wrote the manuscript. All authors read and approved the manuscript.

Funding

This work was funded by the Public Welfare Research and Capacity Building Project of Guangdong Province (No. 2016A030303059 and No. 2017A020211008), and the Key Projects of Basic Research and Applied Basic Research of Guangdong Province Normal University (No. 2018KZDXM041)

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

This article does not contain any studies with human participants or vertebrates performed by any of the authors.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Fig. 1 Results of strain identification (a) Colonies of Strain WA4-31 on Gauze's medium. (b) Observation

Results of Strain WA4-31 under Scanning Electron Microscope of 5000×. (c) Observation Results of Strain

WA4-31 under Scanning Electron Microscope of 20000×. (d) Detection of PCR Products of 16S rDNA by

Agarose Gel Electrophoresis. 1、 2: Black control; 3、 4:WA4-31 16S rDNA; 5:2000 DNA marker. (e)

Phylogenetic Tree of Strain WA 4-31. Maximum-likelihood phylogenetic tree based on 16S rDNA gene

sequences showing the positions of strain WA 4-31, the type strains of other *Gordonia* and representatives of

some other related taxa. Only bootstrap values (expressed as percentages of 1,000 replications) greater than

50% are shown at branching points. Bar, 0.02 substitutions per nucleotide position.

Fig. 2 The antibacterial activity of different fractions against *Candida albicans* with Oxford Cup

method (a) The 3rd and the 4th fractions had anti-*Candida albicans* activity, (b) while the 5th and

the 6th did not, (c) The 10th fraction had stronger antibacterial activity. 1: Positive drug:

Amphotericin B, 32 µg/mL; 2: Blank control: methanol solution; 3-7: Methanol solutions with

different fractions, 5mg/mL.

Fig. 3 Molecular Structure of compound 1-4 (a) compound 1: Actinomycin D. (b) compound 2:

Actinomycin X₂. (c)compound 3: Mojavensin A. (d)compound 4: cyclic (leucine-leucine)

dipeptide

Fig. 4 Activity of various samples against *Candida albicans* by Filter paper method 1: Positive

drug: Amphotericin B, 32 µg/mL; 2: Blank control: methanol solution; 3:the tenth fraction

dissolved in methanol, 1mg/mL; 4: Mojavensin A dipeptide dissolved in methanol, 512 µg/mL; 5:

Cyclic (leucine-leucine) dipeptide dissolved in methanol, 512 µg/mL; 6: Mojavensin A: cyclic

(leucine-leucine) dipeptide =2: 1; 7: Mojavensin A: cyclic (leucine-leucine) dipeptide =1: 1; 8:

Mojavensin A: cyclic (leucine-leucine) dipeptide =1: 2.

Fig. 5 The cytotoxicity (MTT ASSAY) results of compounds on HepG-2cells (a) and CNE-2 cells(b) at the different concentrations for 24-72 h

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Table 1 The Inhibition Zone size of the fractions for *Candida albicans* with Oxford cups method

	3 rd fraction	4 th fraction	10 th fraction	11 th fraction	15 th fraction	16 th fraction	Positive drug
Diameter of the inhibition zone (mm) (n=3, means± SD)							
<i>Candida albicans</i> ATCC 10231	13.0±1.5	10.5±1.0	36.0±2.0	16.0±1.0	13.5±1.0	12.0±1.5	15.0±1.0

Table 2 The Minimal inhibitory concentration (MIC) of Compounds against the tested strains (µg/mL)

	Compound 1 Actinomycin D	Compound 2 Actinomycin X2	Compound 3 Mojavensin A	Compound 4 cyclic (leucine-leucine) dipeptide
<i>Candida albicans</i> ATCC 10231	32	128	64	>256
<i>Trichophyton rubrum</i> ATCC 60836	128	128	16	>256
<i>Aspergillus niger</i> ATCC 16404	128	128	32	128
<i>Aspergillus fumigatus</i> ATCC 96918	128	128	64	>256
MRSA ATCC43300	0.25	0.25	>256	>256
<i>S.aureus</i> ATCC25923	64	128	>256	>256
<i>E.coli</i> ATCC 25922	64	128	32	32
<i>K.pneumoniae</i> ATCC 13883	64	128	>256	>256

Table 3 The Inhibition Zone size of various samples against *Candida albicans* by Filter paper method

	compound 3 Mojavensin A	compound 4 cyclic (leucine-leuci ne) dipeptide	Mojavensin A: cyclic (leucine- leucine) dipeptide =1: 1	Mojavensin A: cyclic (leucine- leucine) dipeptide =2: 1	Mojavensin A: cyclic (leucine- leucine) dipeptide =1: 2	The tenth fraction	Positive drug
	Diameter of the inhibition zone (mm) (n=3, means± SD)						
<i>Candida albicans</i> ATCC 10231	8.5±0.5	-	14.5±1.0	11.5±1.5	8.0±1.5	19.0±1.0	11.5±0.5

Table 4 The half-maximal inhibitory concentration (IC₅₀) of compounds on HepG-2 and CNE-2 cells for 24-72 h (µg/mL)

	HepG-2			CNE-2		
	24h	48h	72h	24h	48h	72h
Actinomycin D	>200	29.74	12.16	>200	15.02	1.24
Actinomycin X₂	>200	14.68	5.37	>200	84.38	0.93
Mojavensin A	-	>200	>200	>200	130.72	51.03

Figure 1

Figure 2

The antibacterial activity of different fractions against *Candida albicans* with Oxford Cup method (a) The 3rd and the 4th fractions had anti-*Candida albicans* activity, (b) while the 5th and the 6th did not, (c) The 10th fraction had stronger antibacterial activity. 1: Positive drug: Amphotericin B, 32 $\mu\text{g/mL}$; 2: Blank control: methanol solution; 3-7: Methanol solutions with different fractions, 5mg/mL.

Figure 3

Molecular Structure of compound 1-4 (a) compound 1: Actinomycin D. (b) compound 2: Actinomycin X2. (c) compound 3: Mojavensin A. (d) compound 4: cyclic (leucine-leucine) dipeptide

Figure 4

Activity of various samples against *Candida albicans* by Filter paper method 1: Positive drug: Amphotericin B, 32 $\mu\text{g/mL}$; 2: Blank control: methanol solution; 3: the tenth fraction dissolved in methanol, 1 mg/mL; 4: Mojavensin A dipeptide dissolved in methanol, 512 $\mu\text{g/mL}$; 5: Cyclic (leucine-leucine) dipeptide dissolved in methanol, 512 $\mu\text{g/mL}$; 6: Mojavensin A: cyclic (leucine-leucine) dipeptide = 2: 1; 7: Mojavensin A: cyclic (leucine-leucine) dipeptide = 1: 1; 8: Mojavensin A: cyclic (leucine-leucine) dipeptide = 1: 2.

Figure 5

The cytotoxicity (MTT ASSAY) results of compounds on HepG-2cells (a) and CNE-2 cells(b) at the different concentrations for 24-72 h

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