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Research Article

Antibacterial potential of rice-associated *Acremonium* sp. USSc24: Bioautography-guided fractionation and GC–MS profiling against drug-resistant *Staphylococcus aureus*

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ABSTRACT

The rapid emergence of antibiotic-resistant *Staphylococcus aureus* strains poses a significant public health challenge, highlighting the need for novel antimicrobial agents. Filamentous fungi are prolific producers of bioactive secondary metabolites, yet many fungal taxa remain underexplored. In this study, ten fungal strains isolated from brown rice were cultured in Czapek–Dox broth and yeast malt broth to prepare crude extracts, which were screened for antibacterial activity against methicillin-sensitive (MSSA), methicillin-resistant (MRSA), vancomycin-intermediate (VISA), and vancomycin-resistant (VRSA) *S. aureus* isolates. Most extracts exhibited weak to moderate activity, whereas *Acremonium* sp. USSc24 displayed striking and consistent antibacterial effects, particularly in extracts produced from yeast malt broth. Disc diffusion and minimum inhibitory concentration assays confirmed broad-spectrum activity against MSSA, MRSA, and VISA strains, with no detectable inhibition of VRSA. Bioautography-guided fractionation localized antibacterial activity to a specific chromatographic fraction, and gas chromatography–mass spectrometry (GC–MS) analysis identified a chemically diverse mixture of compounds, including caryophyllene oxide, tetradecanamide, octadecenamide, and other sesquiterpenes, many of which have reported antimicrobial properties. These results suggest that the observed antibacterial activity is likely due to synergistic interactions among multiple secondary metabolites. Collectively, this study highlights *Acremonium* sp. USSc24 as a promising source of antibacterial compounds and provides a foundation for future isolation, structural elucidation, and *in vivo* evaluation of bioactive metabolites against drug-resistant *S. aureus*.

Keywords:
Acremonium; Antibacterial; Fungal metabolite; Rice-associated fungi

1. INTRODUCTION

The rapid emergence and global dissemination of antibiotic-resistant bacteria represent a major threat to public health and have significantly compromised the effectiveness of existing antimicrobial therapies[1,2]. Among these pathogens, *Staphylococcus aureus* remains one of the most important causes of both community- and hospital-acquired infections, ranging from mild skin and soft tissue infections to life-threatening conditions such as pneumonia, endocarditis, and sepsis[3]. The widespread occurrence of methicillin-resistant *S. aureus* (MRSA), along with the increasing prevalence of vancomycin-intermediate (VISA) and vancomycin-resistant (VRSA) strains, has severely limited treatment options and underscores the urgent need for novel antibacterial agents with new modes of action[4,5].

Filamentous fungi are widely recognized as prolific producers of secondary metabolites with diverse biological activities, including antibacterial, antifungal, and anticancer properties[6,7]. In recent years, research attention has expanded beyond well-characterized fungal

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genera to include less explored taxa as promising reservoirs of novel bioactive compounds. In antimicrobial drug discovery, fungi are capable of synthesizing a broad spectrum of secondary metabolites, such as polyketides, terpenoids, alkaloids, and peptides, many of which exhibit potent antimicrobial activity. Consequently, continued exploration of fungal biodiversity, particularly from unique and underinvestigated ecological niches, remains essential for the discovery of new antibacterial scaffolds. The genus *Acremonium* comprises a diverse group of filamentous fungi commonly found in soil, plant tissues, plant debris, and food-associated environments[8]. Members of this genus are well recognized for their ability to biosynthesize a wide range of biologically active secondary metabolites, including peptides, polyketides, and terpenoids[9,10]. Notably, cephalosporin C—the precursor of modern cephalosporin antibiotics—was originally isolated from *Acremonium chrysogenum*, underscoring the substantial pharmaceutical relevance of this genus[11]. In addition to cephalosporins, *Acremonium* species have been reported to produce other antimicrobial compounds such as pyrrocidines, acremonidins, acremoxanthones, and cyclic peptides[12-14]. Despite these advances, the antibacterial potential of many *Acremonium* species remains insufficiently characterized, particularly against contemporary drug-resistant pathogens such as *Staphylococcus aureus*.

Rice-associated fungi represent an ecologically relevant yet relatively unexplored source of antimicrobial metabolites. Microorganisms inhabiting food matrices often face intense microbial competition, which may drive the production of bioactive compounds as survival mechanisms. However, systematic studies on the antibacterial activity of rice-derived fungi remain limited, leaving their biotechnological potential largely untapped. In this study, crude extracts from ten fungal strains isolated from brown rice were evaluated against a panel of *Staphylococcus aureus* strains. Among these, *Acremonium* sp. USSc24 exhibited the most promising antibacterial activity and was selected for further characterization, including bioautography-guided fractionation and gas chromatography–mass spectrometry (GC–MS) analysis. Collectively, these findings highlight the antibacterial potential of rice-associated *Acremonium* sp. and provide a foundation for future studies on fungal-derived compounds targeting antibiotic-resistant *S. aureus*.

2. MATERIALS AND METHODS

2.1. Microbial cultures

Strains of *Staphylococcus aureus* used in this study included methicillin-sensitive *S. aureus* (MSSA; CRP32, CRP40, and CRP55), methicillin-resistant *S. aureus* (MRSA; CRK01, CRO01, CRO10 CRP35, CRP44, CRP45, CRP46, and CRP47), vancomycin-intermediate *S. aureus* (VISA; CRP57), and vancomycin-resistant *S. aureus* (VRSA; CRP41), as previously described [15]. The reference strain *S. aureus* TISTR 1466 was obtained from the Thailand Institute of Scientific and Technological Research (TISTR), Pathum Thani, Thailand. Ten fungal strains—*Acremonium* sp., *Aspergillus niger*, *Bipolaris* sp., *Curvularia* sp., *Drechslera* sp., *Geotrichum* sp., *Lasiodiplodia theobromae*, *Nigrospora oryzae*, *Penicillium* sp., and *Phoma* sp.—were isolated from brown rice[16]. Bacterial cultures were routinely grown in brain heart infusion (BHI) medium at 37°C for 18–24 h, while fungal strains were cultivated on potato dextrose agar (PDA) at 30°C for 7 days. For short-term storage, bacterial and fungal cultures were maintained at 4 °C on nutrient agar and PDA slants, respectively.

2.2. Preparation of the fungal crude extracts

Ten mycelial discs (6 mm diameter) from one-week-old fungal cultures were transferred into 500 mL of the liquid medium, and incubated at 30°C for one month with shaking (160rpm). Two different culture media were used: Czapek–Dox broth (CDB) and yeast malt broth (YMB). After incubation, fungal mycelia were removed by filtration through Whatman No. 1 filter paper. The culture filtrates were extracted with an equal volume of ethyl acetate using a separatory funnel. The organic phase was collected and concentrated under reduced pressure using a rotary evaporator (EYELA N-1000) to obtain approximately 2 mL of crude extract. The resulting extracts were directly used for subsequent antibacterial assays.

2.3. Antibacterial assay

The antibacterial activity of fungal crude extracts was evaluated using the disc diffusion method [17]. Test bacteria were grown in BHI broth at 37°C for 16–18 h to achieve a final concentration of approximately 1×10^8 CFU/mL. Mueller–Hinton agar plates were swabbed uniformly with the bacterial suspensions. Sterile paper discs were impregnated with 10 µL of fungal crude extract and placed on the inoculated agar surfaces. Plates were incubated at 37°C for 24 h, and antibacterial activity was assessed by measuring the diameter of inhibition zones (mm). Ethyl acetate was used as a negative control. The minimum inhibitory concentration (MIC) was determined using a two-fold serial dilution of the crude extracts. The original crude extract was defined as 1 unit (1 U) and diluted from 1/2 to 1/2048 U. MIC values were determined using the disc diffusion method, and the lowest extract concentration showing visible inhibition

of bacterial growth was recorded as the MIC. The antibacterial efficacy of the crude extracts was compared with standard antibiotic discs, including ampicillin (10 µg), bacitracin (10 U), chloramphenicol (30 µg), erythromycin (15 µg), kanamycin (30 µg), methicillin (5 µg), penicillin (10 U), streptomycin (10 µg), and tetracycline (30 µg), following CLSI guidelines (CLSI 2024). All experiments were conducted in triplicate, and results represent the mean of at least three independent experiments.

2.4. Bioautography

Fungal crude extracts were subjected to thin-layer chromatography (TLC) to separate biologically active compounds. Extracts were applied to silica gel TLC plates and developed using a solvent system of diethyl ether: hexane (9:1, v/v). The developed plates were visualized under ultraviolet (UV) light at 254 and 365 nm. Agar overlay bioautography was performed to locate antibacterial compounds on the TLC plates. Briefly, *S. aureus* cultures (MRSA: CRK01 and CRP45) were grown in BHI broth at 37°C for 16–18 h to obtain a concentration of 1×10^8 CFU/mL and then uniformly spread onto Mueller–Hinton agar plates. The developed TLC plates were placed directly onto the bacterial lawn and incubated at 37°C for 24 h. Zones of inhibition corresponding to active compounds were marked, and retention factor (Rf) values were calculated.

2.5. GC-MS analysis of the active fractions

The bioactive fractions identified by bioautography were analyzed using gas chromatography–mass spectrometry (GC–MS). Analysis was performed on an Agilent 6890 gas chromatograph coupled with an Agilent 5973 mass spectrometer (Agilent Technologies, USA), equipped with an HP-5MS capillary column (30 m × 0.25 mm, film thickness 0.25 µm). Compound identification was achieved by comparing mass spectra with reference spectra in the National Institute of Standards and Technology (NIST) library, allowing determination of compound identity, molecular weight, and structural characteristics.

3. RESULTS

3.1. Prescreening of fungal crude extracts for antibacterial activity

All ten fungal strains—*Acremonium* sp., *Aspergillus niger*, *Bipolaris* sp., *Curvularia* sp., *Drechslera* sp., *Geotrichum* sp., *Lasiodiplodia theobromae*, *Nigrospora oryzae*, *Penicillium* sp., and *Phoma* sp.—were cultured separately in both Czapek–Dox broth (CDB) and yeast malt broth (YMB) to prepare fungal crude extracts. The resulting extracts were subjected to prescreening for antibacterial activity against *Staphylococcus aureus* strains. The prescreening assay revealed that most fungal crude extracts exhibited varying degrees of anti-*S. aureus* activity; however, these effects were generally weak to moderate (data not shown). In contrast, crude extracts derived from *Acremonium* sp. demonstrated striking and consistent antibacterial activity against *S. aureus*. Based on these preliminary results, the *Acremonium* sp. isolate, designated strain USSc24, was selected for further detailed investigation (Figure 1).

3.2. Antibacterial activity and MIC

Crude extracts of *Acremonium* sp. produced in Czapek–Dox broth (CDB) and yeast malt broth (YMB) were evaluated for antibacterial activity against a panel of *S. aureus* strains, including the reference strain (TISTR 1466), methicillin-sensitive (CRP32, CRP40,

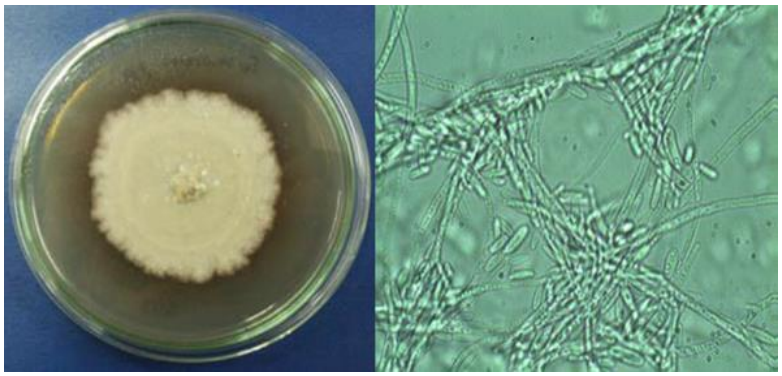

Figure 1. Colony and microscopic morphology of *Acremonium* sp. USSc24. Colonies grown on potato dextrose agar (PDA) for 7 days at 30 °C, showing typical texture and coloration (left). Light microscopy of conidiophores and conidia, highlighting the characteristic hyaline, septate hyphae and conidial structures (right).

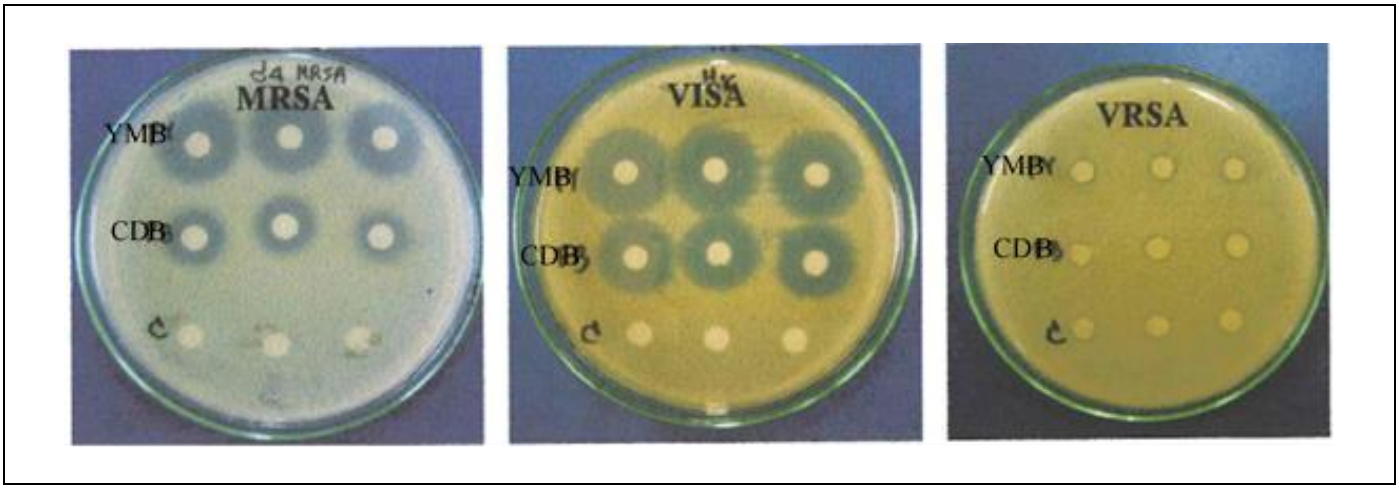

Figure 2. Antibacterial activity of *Acremonium* sp. USSc24 crude extracts against drug-resistant *Staphylococcus aureus* strains. Inhibition zones produced by crude extracts derived from Czapek–Dox broth (CDB) and yeast malt broth (YMB) were assessed using the disc diffusion assay. Representative images show activity against methicillin-resistant (MRSA), vancomycin-intermediate (VISA), and vancomycin-resistant (VRSA) *S. aureus* isolates. Ethyl acetate had no antibacterial activity.

and CRP55), methicillin-resistant (CRK01, CRO01, CRO10, CRP35, CRP44, CRP45, CRP46, and CRP47), vancomycin-intermediate (CRP57), and vancomycin-resistant (CRP41) isolates, using the disc diffusion assay. Both extracts exhibited inhibitory activity against the reference strain and most clinical isolates, with inhibition zone diameters ranging from 12.3 to 23.3 mm (Figure 2, Table 1). Overall, extracts derived from YMB displayed stronger antibacterial activity than those obtained from CDB. For the reference strain TISTR 1466, inhibition zones of 14.6 ± 1.53 mm and 20.3 ± 1.53 mm were observed for CDB and YMB extracts, respectively. Among MSSA isolates, inhibition zones ranged from 12.3 to 14.6 mm for CDB extracts and from 17.0 to 20.0 mm for YMB extracts. MRSA isolates exhibited comparatively higher susceptibility, with with inhibition zones of 13.1–19.0 mm for CDB extracts and 19.6–23.3 mm for YMB extracts. The VISA isolate CRP57 was also

Table 1. Antibacterial activity of *Acremonium* sp. USSc24 crude extracts against *Staphylococcus aureus* isolates. Inhibition zone diameters (mm) of crude extracts prepared from Czapek–Dox broth (CDB) and yeast malt broth (YMB) were determined using the disc diffusion assay. Values represent mean ± standard deviation of three independent experiments.

	CDB	**YMB**
TISTR 1466	14.60 ± 1.53	20.30 ± 1.53
MSSA		
CRP32	13.80 ± 0.58	20.00 ± 0.00
CRP40	12.30 ± 0.58	17.00 ± 1.00
CRP55	14.60 ± 0.58	19.30 ± 0.58
MRSA		
CRK01	19.00 ± 0.00	23.30 ± 0.58
CRO01	17.30 ± 0.29	21.00 ± 0.00
CRO10	15.00 ± 0.00	20.30 ± 0.58
CRP35	13.10 ± 0.29	19.60 ± 0.58
CRP44	17.00 ± 1.00	21.50 ± 0.87
CRP45	12.30 ± 0.29	19.60 ± 0.58
CRP46	17.60 ± 0.58	21.60 ± 1.15
CRP47	17.60 ± 0.58	22.00 ± 0.00
VISA	18.60 ± 0.58	
CRP57	18.60 ± 0.58	21.10 ± 0.29
VRSA		
CRP41	0.00 ± 0.00	0.00 ± 0.00

MSSA = methicillin-sensitive *Staphylococcus aureus*, MRSA = methicillin-resistant *S. aureus*, VISA = vancomycin-intermediate *S. aureus*, VRSA = vancomycin-resistant *S. aureus*.

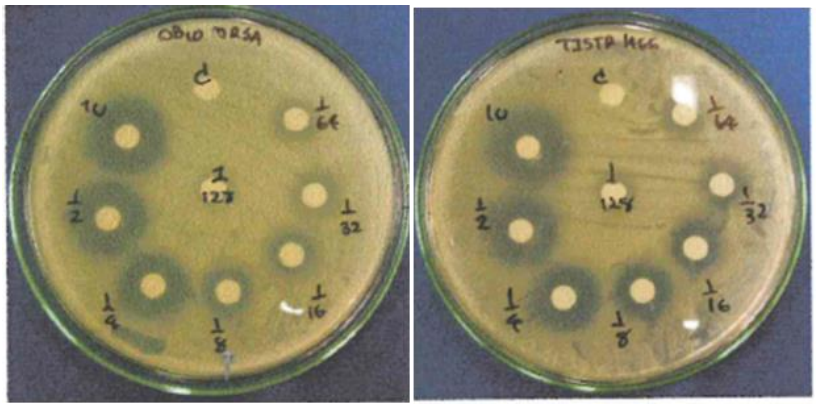

Figure 3. Minimum inhibitory concentrations (MICs) of *Acremonium* sp. USSc24 crude extracts against MRSA (left) and reference (right) strains. Two-fold serial dilutions of crude extracts from were tested using the disc diffusion method. The inhibition zones indicate a concentration-dependent antibacterial effect, with higher concentrations producing larger zones of growth inhibition.

susceptible, with inhibition zones of 18.6 ± 0.58 mm (CDB) and 21.1 ± 0.29 mm (YMB). In contrast, no antibacterial activity was detected against the VRSA isolate CRP41 with either extract. These findings demonstrate that *Acremonium* crude extracts possess broad antibacterial activity against *S. aureus*, particularly against MSSA, MRSA, and VISA strains, with enhanced activity observed for extracts produced in YMB.

The minimum inhibitory concentrations (MICs) of selected *Acremonium* crude extracts were further evaluated against *S. aureus* isolates CRO10, CRP45, and the reference strain TISTR 1466 using a two-fold serial dilution disc diffusion method (Figure 3, Table 2). At the highest concentration (1 U), strong antibacterial activity was observed, with inhibition zones ranging from 21.0 ± 1.15 mm to 22.5 ± 0.58 mm. Pronounced activity was maintained at 1/2 U, with inhibition zones between 20.0 and 21.0 mm. Moderate inhibition was observed at 1/4 and 1/8 U (13.0–18.0 mm), while activity decreased markedly at 1/16 and 1/32 U. No inhibition was detected at 1/128 U for any tested strain. These results indicate a clear concentration-dependent antibacterial effect of the *Acremonium* crude extracts against *S. aureus*.

3.3. Comparison with antibiotics

The antibacterial efficacy of *Acremonium* crude extracts produced in yeast malt broth was compared with selected commercial antibiotics against four *S. aureus* isolates (CRK01, CRO01, CRP35, and CRP44) using the disc diffusion method (Table 3). The crude extracts demonstrated strong inhibitory activity, with inhibition zones ranging from 19.6 ± 0.58 mm to 23.3 ± 0.58 mm. Against isolate CRK01, the fungal crude extracts exhibited comparable or superior activity to most antibiotics tested, including erythromycin and methicillin. For isolate CRO01, the extracts showed substantial inhibition, exceeding the activity of several antibiotics—namely ampicillin, erythromycin, kanamycin, methicillin, and penicillin—which showed no detectable activity against this strain. In the case of CRP35, the crude extracts demonstrated moderate activity, comparable to or greater than most antibiotics

Table 2. Minimum inhibitory concentrations (MICs) of *Acremonium* sp. USSc24 crude extracts against *Staphylococcus aureus* strains. Two-fold serial dilutions of crude extracts from yeast malt broth (YMB) were tested using the disc diffusion method. Inhibition zone diameters (mm) are shown for MRSA isolates (CRO10, CRP45) and the reference strain (TISTR 1466), indicating a concentration-dependent antibacterial effect. Values represent the mean ± standard deviation of three independent experiments.

MIC (U)	CRO10	CRP45	TISTR 1466
1	21.50 ± 1.73	21.00 ± 1.15	22.50 ± 0.58
1/2	21.00 ± 1.53	20.00 ± 1.15	20.00 ± 0.58
1/4	17.00 ± 1.00	17.50 ± 1.00	18.00 ± 0.58
1/8	15.00 ± 0.58	14.00 ± 0.58	13.00 ± 1.00
1/16	10.00 ± 0.58	10.00 ± 0.58	11.50 ± 0.58
1/32	9.00 ± 0.00	8.50 ± 0.58	10.50 ± 0.58
1/64	8.00 ± 0.00	8.00 ± 0.58	8.00 ± 0.00
1/128	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Table 3. Comparative antibacterial activity of *Acremonium* sp. USSc24 crude extracts and selected antibiotics against *Staphylococcus aureus*. Inhibition zones (mm) of crude extracts produced in yeast malt broth (YMB) were compared with those of standard antibiotics against four *S. aureus* isolates (CRK01, CRO01, CRP35, and CRP44) using the disc diffusion assay. Values represent mean ± standard deviation of three independent experiments.

	CRK01	CRO01	CRP35	CRP44
Acremonium YMB extracts	23.30 ± 0.58	20.30 ± 0.58	19.60 ± 0.58	19.60 ± 0.87
Ampicillin (10 µg)	16.00 ± 1.00	0.00 ± 0.00	7.00 ± 0.58	8.00 ± 0.29
Bacitracin (10 U)	19.50 ± 1.15	26.00 ± 0.58	17.50 ± 0.29	14.50 ± 0.58
Chloramphenicol (30 µg)	0.00 ± 0.00	22.00 ± 0.58	21.00 ± 1.00	12.00 ± 0.29
Erythromycin (15 µg)	23.00 ± 0.58	0.00 ± 0.00	6.50 ± 0.50	9.00 ± 0.29
Kanamycin (30 µg)	21.00 ± 0.58	0.00 ± 0.00	6.50 ± 0.29	7.00 ± 0.58
Methicillin (5µg)	23.00 ± 0.29	0.00 ± 0.00	20.00 ± 1.00	7.00 ± 0.58
Penicillin (10 U)	15.50 ± 0.58	0.00 ± 0.00	6.50 ± 0.50	9.00 ± 0.58
Streptomycin (10 µg)	15.50 ± 1.00	14.00 ± 0.58	6.50 ± 1.15	7.00 ± 0.29
Tetracycline (30 µg)	11.50 ± 1.00	11.00 ± 0.58	6.50 ± 0.50	13.00 ± 0.50

except chloramphenicol and methicillin. Similarly, for CRP44, the extract exhibited stronger inhibitory activity than most tested antibiotics. Overall, the *Acremonium* crude extracts showed broad and consistent antibacterial activity, in several instances comparable to or exceeding that of standard antibiotics against resistant *S. aureus* isolates.

3.4. Bioautography analysis

Acremonium crude extracts were fractionated by thin-layer chromatography (TLC) using a diethyl ether:hexane (9:1, v/v) solvent system. Separated compounds were visualized under ultraviolet (UV) light at 254 and 365 nm, as well as by iodine vapor staining (Figure 4). Under UV light at 254 nm, a prominent band with an Rf value of 0.50 was observed, along with additional bands at Rf values of 0.25, 0.48, and 0.83. Iodine vapor visualization revealed two additional compounds with Rf values of 0.51 and 0.60. The antibacterial activity of the separated fractions was evaluated using agar overlay bioautography against two MRSA strains (CRK01 and CRP45). A distinct fraction corresponding to an Rf value of 0.60 exhibited clear zones of bacterial growth inhibition (Figure 4), indicating the presence of bioactive antibacterial compound(s) within this fraction.

3.5. GC–MS profiling

GC–MS analysis of the bioactive *Acremonium* fraction revealed a diverse chemical composition (Table 4).

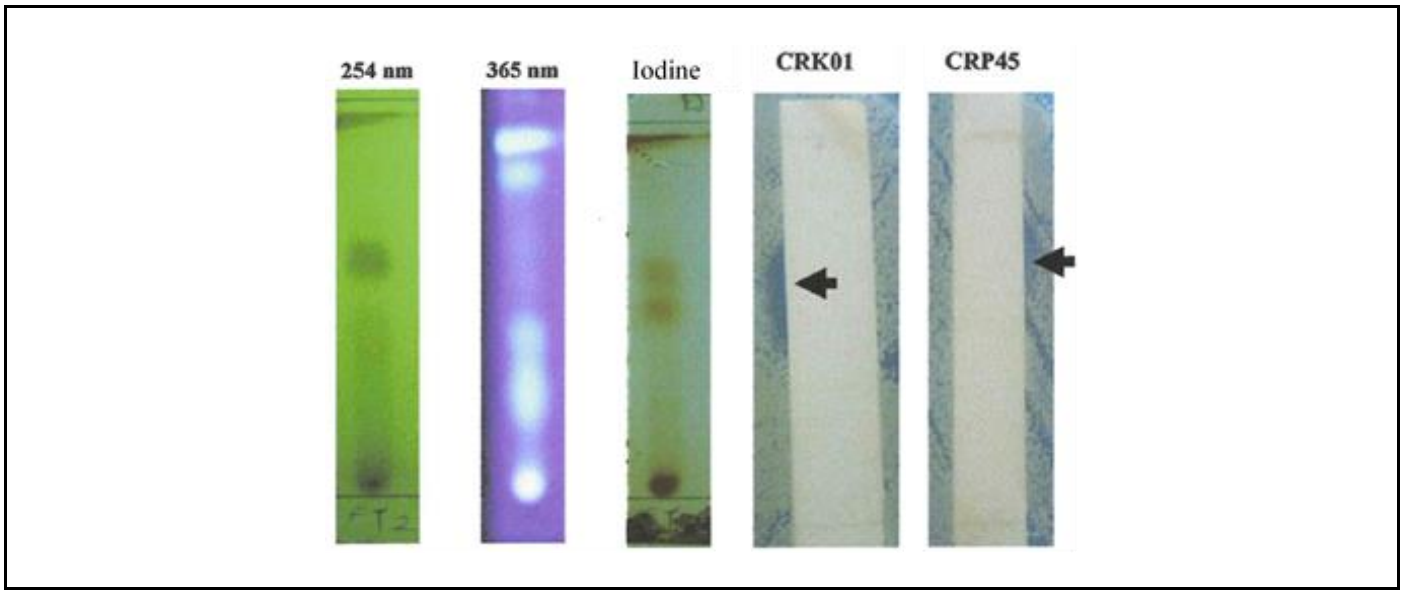

Figure 4. Thin-layer chromatography (TLC) and bioautography of *Acremonium* sp. USSc24 crude extracts. TLC profiles of the crude extracts developed using diethyl ether:hexane (9:1, v/v) and visualized under UV light (254 and 365 nm) and iodine vapor, showing multiple separated bands. Bioautogram of the TLC plate overlaid with MRSA strains (CRK01 and CRP45), highlighting the fraction with an Rf value of ~0.60 that exhibited clear antibacterial activity.

Table 4. Chemical composition of *Acremonium* sp. USSc24 crude extracts as determined by GC–MS analysis. Compounds were identified from crude extracts using gas chromatography–mass spectrometry, with retention times (min) and relative peak areas (%) reported.

Chemicals	RT (min)	Area (%)
Caryophyllan-2,6 beta oxide	16.95	3.21
Neoalloocimene	17.95	1.16
Calamenene	19.46	1.08
4,8-dimethyl-nona-3,8-dien-2-one	20.64	0.98
Caryophyllene oxide	20.942	9.56
1-(1,1-dimethylsthyl)-2-methyl-1,3-propanediyl ester-2-methyl-propanoic acid	21.16	0.77
Alloaromadendrone	21.41	0.88
Humalene-1,2-epoxide	21.54	1.90
1,4-dimethyl-7-(1-hydroxy-1-methyl-ethyl)-4,5,6,7-[3H]hexahydroazulene	22.50	1.22
Caryophylla-3,8(13)-dien-5.beta.-oledol	22.65	1.23
Isoaromadendrene epoxide	22.97	2.53
5-methyl-2(2-propenyl)-1,3-cyclohexadione	23.20	2.59
4-methyl-cyclohex-3-ethyl-methano	23.65	6.42
2,2,6,7-tetramethyl-10-oxatricyclo[4.3.0.1(1,7)]decan-5-ol	24.38	1.09
Longifolenaldehyde	25.09	0.72
2,2,6,8-tetramethyl-7-oxatricyclo[6.1.0.0(1,6)]nonane	25.16	0.69
Octahydro-4,8,8,9-tetramethyl-1,4-methanoazulen-7(1H)-one	25.95	4.88
2,6,6-trimethyl-1-cyclohexene-1-butyraldehyde	26.10	3.00
3-methoxy-2,5-dimethylpyrazine	26.70	1.84
4,4,8-trimethyltricyclo[6.3.1.0(1,5)]dodecane-2,9-diol	27.31	0.45
Furasano(3,4-d)pyrimidine-5,7(4H,6H)-dione	28.31	4.03
(4aS,5R,8R)-5-methyl-8-[5-(trimethylsilyl)-4-pentynyl]-2,3,4,4a,5,6,7,8-octahexahydropyridol[1,2-b][1,2]oxazine	28.56	1.29
Bis(2-methylpropyl)phynyl-phosphine	29.17	3.20
Hexadecenenitrile	31.11	1.11
9-octadecenamide	32.58	1.23
Tetradecanamide	32.98	9.72
Triacontane	33.12	1.46
Octadecenamide	36.99	3.47
Hexacosane	37.13	0.52

A total of 30 compounds were identified, with retention times ranging from 16.95 to 37.13 min. The major constituents included tetradecanamide (9.72%), caryophyllene oxide (9.56%), 4-methyl-cyclohex-3-ethyl-methano (6.42%), octahydro-4,8,8,9-tetramethyl-1,4-methanoazulen-7(1H)-one (4.88%), and furasano(3,4-d)pyrimidine-5,7(4H,6H)-dione (4.03%). Several additional compounds, including caryophyllan-2,6-beta-oxide, octadecenamide, and other sesquiterpenes and fatty acid derivatives, were detected at lower relative abundances. The chemical diversity revealed by GC–MS analysis suggests that multiple constituents may collectively contribute to the observed antibacterial activity of the *Acremonium* crude extracts.

4. DISCUSSION

The present study demonstrates that crude extracts from rice-associated fungi exhibit varying antibacterial activity against *Staphylococcus aureus*. Among ten fungal isolates, *Acremonium* sp. (USSc24) showed the most pronounced and consistent inhibitory effects, warranting further investigation. Our data revealed that *Acremonium* extracts displayed striking inhibition, consistent with reports of this genus as prolific producers of bioactive compounds[9,10]. Known metabolites from *Acremonium*, including acremonidins, acremoxanthones, cyclic hexapeptides, and pyrrocidines, possess potent antibacterial properties[12–14]. Notably, the extracts exhibited broad-spectrum activity against methicillin-sensitive (MSSA), methicillin-resistant (MRSA), and vancomycin-intermediate (VISA) *S. aureus*, but not vancomycin-resistant (VRSA) strains, likely due to cell wall modifications conferring cross-resistance[19]. The strong inhibition of MRSA and VISA strains is particularly relevant in the context of global antibiotic resistance. Culture conditions influenced metabolite production, as extracts from yeast malt broth

(YMB) consistently showed stronger antibacterial activity than those from Czapek–Dox broth (CDB), likely due to richer carbon and nitrogen sources promoting secondary metabolism[20,21]. MIC assays demonstrated dose-dependent activity, supporting the presence of active antimicrobial constituents rather than nonspecific effects. Compared with commercial antibiotics, the YMB crude extract showed comparable or superior activity against resistant *S. aureus*, including strains resistant to β-lactams and macrolides[14,22]. Bioautography-guided fractionation localized antibacterial activity to a TLC fraction with an Rf of 0.60, providing a basis for future purification and structural elucidation[23]. GC–MS profiling revealed a chemically diverse mixture, including compounds with known antimicrobial properties. Caryophyllene oxide, a major constituent, has demonstrated activity against *S. aureus*, *E. coli*, and fungi, likely via membrane disruption[24]. Fatty acid amides such as tetradecanamide and octadecenamide exhibit antimicrobial, antibiofilm, and membrane-active effects[25]. Additional sesquiterpene-related compounds, including caryophyllan-2,6-beta-oxide and octahydro-4,8,8,9-tetramethyl-1,4-methanoazulen-7(1H)-one, may contribute synergistically to antibacterial activity[26]. Importantly, the combined use of bioautography and GC–MS in this study offers a complementary approach: bioautography identifies the location of biological activity on the chromatogram, whereas GC–MS provides preliminary insight into the chemical constituents present within that active region. Nevertheless, this linkage should be interpreted with caution. GC–MS primarily detects volatile and thermally stable compounds and does not directly confirm which of the detected constituents are responsible for the observed antibacterial activity. In addition, the presence of multiple compounds within the active fraction suggests that the inhibitory effect may arise from synergistic interactions rather than a single dominant molecule, a phenomenon commonly observed in natural product extracts.

Although the results are promising, several limitations should be acknowledged. First, the chemical analysis is not comprehensive. GC–MS was applied as an initial screening tool, but it mainly detects volatile and thermally stable compounds. Many fungal antimicrobial metabolites (e.g., peptides, polyketides, and β-lactam antibiotics) are non-volatile and therefore may not be detected by this method. As a result, the compounds identified here may represent only part of the metabolite profile, and the main bioactive agents could have been missed. Second, the fungal isolate has not been fully characterized at the molecular level. It was designated as *Acremonium* sp. based on morphological information provided by the source[16]; however, morphology alone is often inadequate for accurate fungal identification. Because the original strain was not available, the molecular analysis (e.g., sequencing of the ITS or LSU rRNA regions) could not be carried out, limiting the precision of the taxonomic assignment. Future work should focus on bioassay-guided fractionation and detailed structural analysis using techniques such as LC–MS/MS and NMR. In addition, proper preservation of isolates together with molecular identification will be important to ensure accurate classification and reproducibility in subsequent studies.

5. CONCLUSIONS

In conclusion, *Acremonium* sp. USSc24 is a promising source of secondary metabolites with notable *in vitro* activity against MSSA, MRSA, and VISA strains, with stronger effects observed in YMB extracts. Bioautography localized antibacterial activity to a TLC fraction (Rf 0.60), and GC–MS provided preliminary chemical profiling, suggesting that multiple compounds may act synergistically. However, the specific bioactive constituents require further exploration. Future studies should focus on bioassay-guided purification, structural elucidation using LC–MS/MS and NMR, and molecular characterization of the fungal isolate to confirm its identity, assess cytotoxicity, and explore mechanism of action. These steps are essential to validate and advance the therapeutic potential of *Acremonium*-derived metabolites.

6. ACKNOWLEDGEMENTS

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Author contribution

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Conflict of interest

None to declare.

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