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ORIGINAL ARTICLE

Isolation and screening of *Thermoactinomycetaceae* family members as an extremophilic poor investigated and promising natural source of antimicrobial substances

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ABSTRACT

Background and Objectives: Recent evidences have shown that methicillin-resistant *Staphylococcus aureus* (MRSA) can cause severe infections and is resistant to almost all commercially available antibiotics. Therefore, screening unknown sources of biological compounds such as the *Thermoactinomycetaceae* family as extremophilic bacteria may be helpful to find new antimicrobial agents.

Materials and Methods: Various samples were collected from different ecosystems, including desert, volcano, compost, and forest. They were cultured on Soil extract agar and Water agar. The antimicrobial activity of the isolates was evaluated using agar overlay and well diffusion methods. Members of the *Thermoactinomycetaceae* family were selected for further study: Their ability to grow at different temperatures, NaCl concentrations, and pH values, enzyme production ability, antimicrobial secondary screening, fractionation of their supernatants and so on.

Results: According to molecular identification of active isolates against MRSA, three strains, including *Laceyella sacchari* UTMC 2705, *Thermoactinomyces* sp. UTMC 2721, and *Laceyella* sp. UTMC 2731, belonged to *Thermoactinomycetaceae* were identified. The minimum inhibitory concentrations of their extracts were tested against some pathogenic bacteria, showing their antimicrobial activity with a broad spectrum. The results of TLC bioautography of the extracts showed that the most active fractions were semi-polar. Also, the results of HPLC analysis showed the existence of several UV-active compounds in their extracts.

Conclusion: The present study highlighted the importance and potential of *Thermoactinomycetaceae* members as a less-known source of antibiotics against pathogenic bacteria.

Keywords: Antibiotic resistance; Bioactive compound; Extremophiles; Isolation; Methicillin-resistant *Staphylococcus aureus*; *Thermoactinomycetaceae*

INTRODUCTION

Before the discovery of antibiotics, infectious diseases were serious causes of mortalities. However, the use of antimicrobial drugs has treated life-threatening infections and extended human life expectancy ~20 years. However, the enormous and irresponsible use of antibiotics in medicine, veterinary and animal husbandry led to the emergence of antimicrobial

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resistant pathogens due to the natural selection process. According to the warning of the World Health Organization (WHO), antibiotic resistance is becoming an urgent problem nowadays (1). MRSA is a major cause of morbidity and mortality, as reported by the centers for disease control and prevention (CDC). It has been shown that the mortality rate of MRSA infections is higher than more than that of AIDS, at 39.9%. The majority of MRSA isolates (81.7%) were multidrug-resistant strains (2, 3).

Microorganisms are one of the most potent natural resources for biological compounds, and ~12000 compounds with antimicrobial activity have been isolated from microbial resources (4). Among bacterial domains, five phyla including: *Actinobacteria*, *Bacteroidetes*, *Cyanobacteria*, *Firmicutes*, and *Proteobacteria* are significant producers of antimicrobial compounds (5).

Many antibiotics have originated from non-extreme terrestrial bacteria. Despite great attention given to bacteria from non-extreme environments, the rate of discovery of new antibiotics from these bacteria has declined over time. Extremophilic microorganisms such as thermophiles, which has been poorly investigated, seems to be good source of novel and effective antibiotics like thermorubin, myriocin and dihydrogranaticin produced by *Thermoactinomyces antibioticus*, *Myriococcum albomyces* and *Streptomyces thermoviolaceus*, respectively (6).

The family *Thermoactinomycetaceae* belongs to order *Bacillales*, class *Bacilli* and phylum *Firmicutes* as a member of the thermophiles. Most strains of this family have been isolated from compost, hay, manure, bagasse, soil and other sources. As of the writing this article (Aug. 2022), there are 25 genera in this family (http://www.bacterio.net). *Staphylospora marina* is one of the new *Thermoactinomycetaceae*, isolated from a deep-sea hydrothermal vent in 2019 (7). Members of the *Thermoactinomycetaceae* are thermophilic, Gram positive, chemoorganotrophic, aerobic, endospore formers with branched substrate and white or yellow aerial mycelia. The peptidoglycan wall contains meso-diaminopimelic acid (meso-DAP) or LL-diaminopimelic acid (LL-DAP). The flat or slightly ridged colonies usually grow rapidly at 55°C on culture media such as ISP2. The genera *Thermoactinomyces* and *Laceyella* have species specific phages. There are some reports of human hypersensitivity pneumonitis caused by spore antigens from members of the *Thermoactinomycetaceae* (8).

Prior to the development of polyphasic taxonomy, the *Thermoactinomycetaceae* were included in the Actinomycetes based on their phenotypic characteristics, such as filamentous growth. However, due to the formation of dipicolinic-acid-containing endospores, low G+C content of DNA, different menaquinone profiles, and similarity of 16SrRNA gene sequences, they were assigned to the phylum *Firmicutes* (8, 9).

Various biotechnological potentials have been described for members of the *Thermoactinomycetaceae*. For example, members of the *Mechercharimyces* family, e.g., *Thermoactinomyces*, *Laceyella* and *Planifilum*, have been considered as biofertilizers. They play an important role in composting, cellulase production, deodorization and mineralization of organic nitrogen. Also, they produced various industrial enzymes, such as serine protease, amylase, thermitase, xylanase, and the enzymes for the degradation of cyclic maltodextrins and polylactate plastic (PLA) (8).

In this study, we focused on the isolation of *Thermoactinomycetaceae* from Iranian sources and investigated their potential as a source of antimicrobial biological compounds against MRSA and some other pathogenic bacteria.

MATERIALS AND METHODS

Sampling and pretreatment. Samples were collected from composts, volcanos, deserts and forests belonging to different geographical locations in Iran. Each sample was collected from 10-15 cm depth from the soil surface and immediately transported to the laboratory. They were air dried, crushed, sieved and pretreated in an oven at 120°C for 10 minutes (10).

The samples were decimally diluted and the corresponding dilutions (10^{-1}, 10^{-2} and 10^{-3}) were cultured on Soil extract agar (17 g agar in 1 L soil extract) and Water agar (17 g agar in 1 L tap water) at pH 7.2 as isolation media. The plates were incubated at 50°C for one week. The morphological colonies resembling to *Thermoactinomycetaceae* were subcultured on ISP medium 2 which consisting of malt (5 g/L), glucose (2 g/L), yeast extract (2 g/L), $CaCO_3$ (1 g/L) and agar (17 g/L) as a maintenance medium (8). Tetracycline (50 µg/mL) was added to exclude contamination by other fast growing *Bacillus* strains. The purified isolates were deposited in the University of Tehran Microor-

ganisms Collection (UTMC).

Resistance verification of MRSA UTMC 1401. *Staphylococcus aureus* UTMC 1401, a methicillin resistant strain (MRSA) was obtained from the University of Tehran Microorganisms Collection (UTMC). Antibiotic resistance was verified by antibiotic susceptibility testing according to Clinical & Laboratory Standards Institute (CLSI) (11). MRSA (5×10^5 CFU/mL) was inoculated and spread on Muller Hinton agar plates. Then, various antibiotic discs including, ciprofloxacin (5 µg), rifampicin (5 µg), cefoxitin (30 µg), tetracycline (30 µg), gentamicin (10 µg), erythromycin (15 µg), clindamycin (2 µg), novobiocin (5 µg), doxycycline (30 µg) and Trimethoprim- sulfamethoxazole (1.25/23.75 µg) were placed on the plate, and incubated at 37°C for 18 hours. The diameter of growth inhibition zones around antibiotic discs was measured. In addition, the MIC of vancomycin against MRSA was measured. This antibiotic was used as a positive control in the following.

Primary screening. Antimicrobial activity of all isolates was evaluated by agar overlay and agar well diffusion methods as described below. Isolates were cultured on ISP2 agar medium and incubated at 50°C for 7 days. Then, a suspension of MRSA (5×10^5 CFU/mL) in sterile melted Muller Hinton broth containing 0.7% agar was poured onto the colonies of isolates on ISP2 agar plate. The plates were incubated overnight at 37°C. The growth inhibition zones against MRSA around the colonies were considered as antimicrobial production by the isolates (12). In addition, all isolates were cultured in 10 mL of ISP2 broth medium consisting of malt (10 g/L), yeast extract (4 g/L), and glucose (4 g/L) and incubated at 50°C, 7 days, 180 rpm. A suspension of MRSA (5×10^5 CFU/mL) was inoculated in the melted Mueller Hinton broth containing 0.7% agar and distributed among the Petri dishes. Six wells were drilled into the inoculated Mueller Hinton agar and filled with the supernatant of the fermentation broth of the isolates. The plates were kept at 4°C for 2 hours to allow the metabolites to diffuse into the medium before MRSA growth. Then the plates were incubated overnight at 37°C. The inhibition zone diameters were expressed in millimeters. The bioassay was performed in duplicate (13).

Molecular identification of active isolates using 16S rRNA gene sequencing. Molecular identification of active isolates against MRSA was performed by PCR amplification of 16S rRNA gene with universal primers (9F (5AAGAGTTTGATCATGGCTCAG-3) and 1541R (5-AGGAGGTGATCCAACCGCA-3) followed by sequencing. They were cultured in BHI broth medium and incubated at 50°C for at least 24 hours. Biomass was isolated by centrifugation at 2300 g for 10 minutes and their genomic DNA was extracted using a DNA extraction kit (Pooya Gene Azma, Iran). The PCR products were sequenced by Macrogen (South Korea). The sequences were blasted in the NCBI and EzTaxon databases. Anti-MRSA active isolates that belonged to the *Thermoactinomycetaceae* family were selected for further studies.

Morphological, physiological and chemotaxonomical characterization. After studying the morphology of the colony, microscopic examinations were performed using the coverslip culture method. For this purpose, sterile coverslips were placed in the ISP2 medium at an angle of 30 degrees, the bacterial cells were inoculated at the insertion site of the coverslip and incubated at 50°C for 7 days. Then the coverslips were Gram stained and viewed with a light microscope (12). The ability of the isolates to grow at different temperatures (28, 37, 50, 55, 60°C), different pH values (3-11) and different NaCl concentrations (2.5, 5, 7.5, 10%) was investigated. The enzymatic activity of the isolates, including the degradation of starch, protein (skim milk) and lipid, was also studied. The enzymatic activity of the isolates was measured based on (diameter of the halo zone diameter of the colony) / diameter of the colony (14, 15). The type of diaminopimelic acid isomer in their cell wall was determined as well (16). In brief, after acid hydrolysis of the biomass, the cell wall amino acids were separated by thin layer chromatography using n-butanol-distilled water-pyridine-toluene (10:6:6: 1, vol/vol) as the mobile phase. Acid aniline phthalate was used to visualize the spots. The R_f value of the spots was compared with that of the controls to determine the type of DAP (meso, LL).

Secondary screening. For further studies, the fermentation broth extracts (FBE) of the active isolates against MRSA UTMC 1401, which belonged to the *Thermoactinomycetaceae* family, were prepared. Briefly, seeding material was prepared by inoculating the appropriate concentration of spore suspension (~10^7-10^8 spores ml^{-1}) in ISP2 broth (50 mL). The

flasks were incubated in a shaker incubator at 180 rpm, 50°C, for 24 hours. The seeding material (7% v/v) was transferred to a 1000 mL Erlenmeyer flask containing 250 mL ISP2 broth as fermentation medium. The fermentation flasks were incubated at 50°C in a shaker incubator at 180 rpm for 2 days. Then, the fermentation broth was centrifuged at 4000 rpm for 10 min and mixed with the same amount of ethyl acetate. The FBEs were obtained after evaporation of the solvent using a rotary evaporator at 37°C (17). The extracts were stored at -20°C until use. The antibacterial activity of three concentrations of the FBEs (50, 100, and 200 µg/well) against MRSA was assayed by the agar well diffusion method as described previously. Methanol and vancomycin (30 µg/well) were used as negative (solvent of the extracts) and positive controls, respectively. The bioassay was performed in two replicates.

The minimum inhibitory concentration of FBEs (10, 5, 2.5, 1.25, 0.625, 0.312, 0.156, 0.078 µg/well) was determined against various pathogenic bacteria, including *Escherichia coli* UTMC 1465, *E. coli* TolC UTMC 1462, *Pseudomonas aeruginosa* UTMC 1463, *Micrococcus luteus* UTMC 1461, *Mucor hiemalis* UTMC 5057, and *Bacillus subtilis* UTMC 1464 using the micro broth dilution method according to CLSI, M7-A09 (11). The MIC was determined as the lowest concentration of the extract that completely inhibited bacterial growth. The bioassay was performed in duplicate. Vancomycin and Muller Hinton broth were used as negative and positive growth controls, respectively. In addition, Muller Hinton broth + methanol was tested to ensure that methanol did not have a lethal effect on the bacteria at the levels used. Sterilized Muller Hinton broth was tested to ensure sterilization.

Brine shrimp lethality bioassay. The brine shrimp bioassay was used to investigate the toxicity of FBEs at different concentrations (200, 100, 50, and 25 µg/ml) against Artemia salina. Briefly, a suspension of nauplii containing 10^{-15} organisms was added to each test sample (4 concentrations) and the covered plate was incubated at 25°C for 24 hours. The plate was examined under the stereoscope and the number of immobile nauplii was counted. Lethality percent of each concentration was calculated according to Apu et al. (18).

Thin-layer chromatography (TLC)–bioautography. Bioautographic evaluation was performed on a TLC plate to demonstrate the antimicrobial activity of the FBEs. The FBEs (800 µg/mL) were spotted onto the TLC plate (Merck, silica gel 60 F254). Then it was developed in a methanol-dichloromethane mixture (1:7) as a mobile phase. To determine the UV active fractions, the developed air-dried TLC plate was observed under two different UV wavelengths (254 nm and 366 nm). To verify the antimicrobial ability of the partially separated biological compounds, the TLC plates were overlaid with an MRSA inoculum with a bacterial count of 5 105 CFU/mL (10 mL) in Muller Hinton broth medium. After solidification of the medium, the TLC plates were incubated at 37°C for 24 hours. Then, the plates were sprayed with an aqueous solution (2.5 mg/mL) of 2,3,5-triphenyltetrazolium chloride (TTC) and incubated at 37°C for 2 hours. Inhibition zones were observed as a transparent area against a red colored background (13).

HPLC of fermentation broth extracts. Crude extracts of selected isolates were analyzed by high-performance liquid chromatography (HPLC)-UV. Reversed-phase HPLC experiments were performed using an XBridge C18 column 100×2.1 mm (Waters), 3.5 µm, solvent A [H_2O-acetonitrile (95/5), 5 mmol NH_4Ac, 0.04 mL/L CH_3COOH]; solvent B [H_2O-acetonitrile (5/95), 5 mmol NH_4Ac, 0.04 mL/L CH_3COOH]; gradient system, 10% B increasing to 100% B in 30 min; flow rate 0.3 mL/min; 40°C.

Column chromatography-bioautography. The FBE with the highest activity was selected for further fractionation by column chromatography. The column was first filled with dry stationary phase powder (powdered silica gel), followed by the addition of the mobile phase, hexane, which was purged through the column until it was completely wetted. The selected extract was placed on top of the column and sponged with the solvent system Hexane: Ethyl acetate 100:0- 95:5- 90:10- 85:15- 80:20- 75:25- 70:30- 65:35- 60:40- 55:45- 50:50- 40:60- 30:70- 20:80- 10:90- 0:100 and finally with methanol to sponge the most polar fractions. Throughout the chromatography process, the eluent is collected in a series of fractions (19). The components were separated by R_f values during visualization under UV light (254 and 366 nm). Thin layer chromatography (TLC) and subsequent bioautography, mentioned in the previous section, were performed to determine the antimicrobial fractions.

RESULTS

Thirty-six isolates were obtained from 54 environmental samples. All *Thermoactinomycetaceae* members were isolated from soil extract agar media. Among the geographical areas, the desert had the highest number of *Thermoactinomycetaceae* family members.

Antimicrobial susceptibility profile of MRSA UTMC 1401. The antimicrobial susceptibility results of MRSA UTMC 1401 (Table 1) showed that the pathogen was resistant not only to methicillin but also to other commonly used antibiotics, including: ciprofloxacin, rifampicin, tetracycline and gentamycin. The MIC ≤ 4 confirmed the susceptibility of this strain to vancomycin as a positive control.

Antimicrobial activity of the isolates. Among all isolates examined, six were: UTMC 2705, UTMC 2717, UTMC 2721, UTMC 2722, UTMC 2731, and UTMC 2735 showed anti-MRSA activity. The results of the two methods of primary screening confirmed each other. The diameters of the growth inhibition zones of their supernatants in the preliminary antimicrobial bioassays were 28, 30, 29, 19, 30, and 27 mm, respectively. Their molecular identification showed that three of them belonged to Actinobacteria and the others to the genera *Laceyella* and *Thermoactinomyces* (Table 2). The results of secondary screening, MIC determination and toxicity of fermentation broth extracts of three selected isolates with anti-MRSA activity (*Laceyella sacchari* UTMC 2705, *Thermoactinomyces* sp. UTMC 2721 and *Laceyella* sp. UTMC 2731) were summarized in Table 3. The Brine shrimp lethality assay showed that the FBE of these three isolates did not exhibit toxicity at concentrations less than 200 µg/ml.

Physiological and chemotaxonomical characteristics. The physiological and chemotaxonomic characteristics of the selected isolates are shown in Table 4. According to the results, all of them had *meso* isomer in their cell wall; they were moderately thermophilic and spore-formers. These fast-growing isolates can tolerate NaCl (2.5% w/v) and grow in a wide pH range (4-10). In addition, they were obtained from compost and desert with good enzymatic activity, due to their role in decomposition in these ecosystems.

Table 2. Molecular identification results

UTMC code	Nearest neighbor	Similarity (%)	Length (bp)
2705	*Laceyella sacchari*	100	735
2721	*Thermoactinomyces daqus*	99.86	888
2731	*Laceyella sacchari*	96.24	801

Thin-layer chromatography (TLC)–bioautography. Considering the mobile and stationary phases used (methanol: dichloromethane (1:7) and silica gel, respectively) for thin layer chromatography, the spots with high retention factor (R_f) showed the presence of compounds with lower polarity than the compounds with lower R_f (Table 5). The most active fractions were semi-polar.

Variety and number of UV-active compounds in the selected FBEs. HPLC analysis showed that several UV-active compounds were present in the fermentation broth extracts of the three selected active isolates UTMC 2705, UTMC 2731 and UTMC 2721 at three wavelengths (210, 350, 360 nm) (Fig. 2).

Column chromatography-bioautography. The FBE of *Laceyella* sp. UTMC 2731 was selected for further fractionation by column chromatography. A total of 24 fractions were collected, most of which were sponged with high polarity methanol. The fractions sponged with the solvent systems Hexane: Ethyl acetate; 60:40- 50:50- 40:60 and pure methanol showed

Table 1. The antimicrobial susceptibility of MRSA UTMC 1401

Antibiotics	Inhibition zone diameter (mm)	Resistant/ susceptible	Antibiotic	Inhibition zone diameter (mm)	Resistant/ susceptible
Ciprofloxacin	0		Erythromycin	27	
Rifampicin	0		Clindamycin	35	
Cefoxitin	0	Resistant	Novobiocin	35	Susceptible
Tetracycline	10		Doxycycline	20	
Gentamycin	0		Trimethoprim	32	

Table 3. Diameter of growth inhibition zones (SD ± 5%) in secondary screening, MIC determination against pathogenic bacteria and mortality rates of three chosen isolates' FBEs (*Laceyella sacchari* UTMC 2705, *Thermoactinomyces* sp. UTMC 2721 and *Laceyella* sp. UTMC 2731).

UTMC code	Concentration/ Test strain	2705	2721	2731
Diameter of growth	50 µg/well	15	12	15
inhibition zone (mm)	100 µg/well	16	15	19
	200 µg/well	20	18	22
MIC (µg/mL)	*Escherichia coli*	>10	>10	>10
	E. coli TolC	2.5	10	10
	Pseudomonas aeruginosa	>10	>10	>10
	Micrococcus luteus	2.5	>10	>10
	Mucor hiemalis	>10	10	5
	Bacillus subtilis	2.5	>10	>10
Brine shrimp	25 µg/mL	0%	0%	0%
mortality rate (%)	50 µg/mL	0%	0%	0%
	100 µg/mL	0%	0%	0%
	200 µg/mL	64%	0%	24%

Table 4. Some physiological and chemotaxonomical characteristics of chosen isolates.

UTMC code	Geographical condition	Temperature (moderately or strictly)	Incubation time	DAP isomer	pH range of growth	NaCl tolerance (% w/v)	Lipase	Protease	Amylase
2705	Compost	Moderately	Fast	Meso	4-10	2.5	-	+	+
2721	Desert	Moderately	Fast	Meso	4-9	2.5	-	+	+
2731	Desert	Moderately	Fast	Meso	4-10	5	-	+	+

(−) No enzymatic activity, weak enzymatic activity (+), mediocre enzymatic activity (++) and enzymatic high activity (+++).
Moderately thermophilic (growing at temperature 28-60°C), strictly thermophilic (growing at 37-65°C) (20)
Incubation time: Fast (one to two days), Mediocre (three to seven days), Slow (more than seven days)

Table 5. Properties of active compounds with anti-MRSA activity in crude extracts of 3 chosen isolates. Active compounds in each crude extract have been shown by S which means created spots on TLC plates.

UTMC code	Anti-MRSA active spots	R_f (cm)	Polarity
2705	S_1	0.02	Polar
	S_2	0.32	Semipolar
	S_3	0.58	Semipolar
2721	S_1	0.07	Polar
	S_2	0.23	Semipolar
	S_3	0.57	Semipolar
	S_4	0.70	Non-polar
2731	S_1	0.52	Semipolar
	S_2	0.61	Non-polar

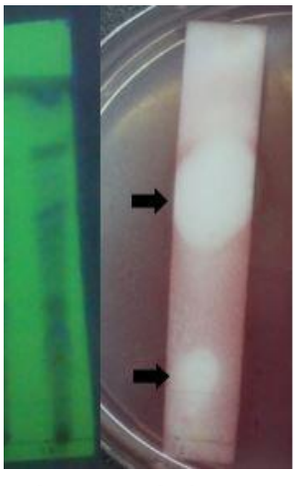

Fig. 1. Thin layer chromatography-bioautography of UTMC 2731 crude extract. Marked spots showed anti-MRSA activity

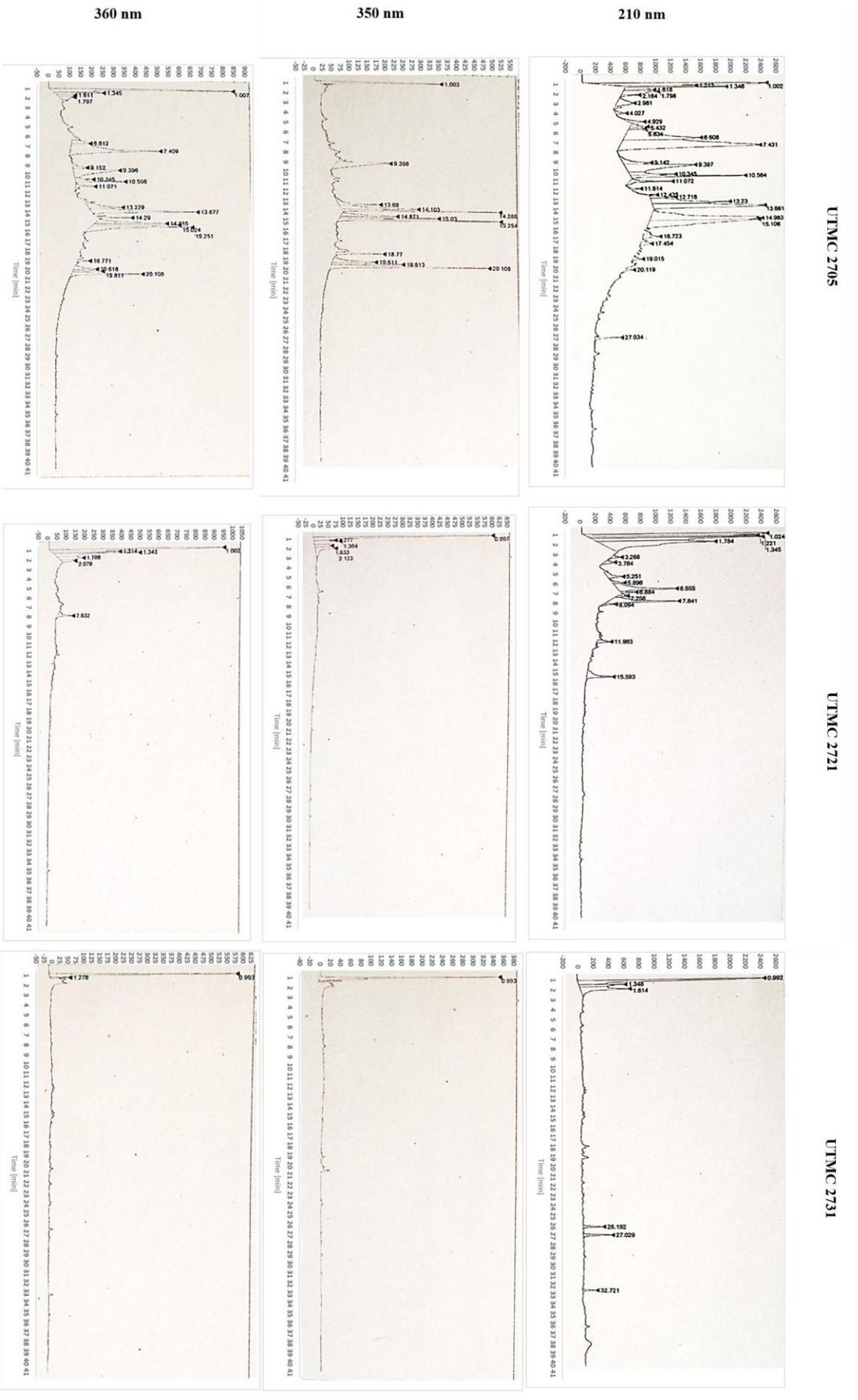

Fig. 2. HPLC results of promising strains at three wavelengths (210, 350, 360 nm)

anti-MRSA activity according to bioautographic results (Fig. 1).

DISCUSSION

MRSA is one of the most worrisome resistant pathogenic bacteria worldwide. Of the two billion healthy people who carry *Staphylococcus aureus*, 2.6% (53 million) of them are infected with MRSA. The mortality rate of MRSA infections ranges from 20% to 50%, highlighting the importance of screening studies to find new and effective therapeutics for this resistant bacterium as a model of pathogenic bacteria (21).

To this end, libraries with abundant bioactive compounds should be screened. The chance of finding novel, effective, sustainable, and broad-spectrum antimicrobial compounds increases with the screening of unexplored sources such as the extremophilic world. On the other hand, they offer unique properties under unadulterated conditions compatible with industrial ones (22).

In the study by Montero and Barrientos, the Antarctic environment was considered as a potential source of novel antibiotic compounds (23). In another study, isolation of Actinobacteria from extreme environments increases the chances of obtaining genera other than *Streptomyces* with novel antibiotics (24). *Paecilomyces lilacinus* and *Nocardiopsis dassonvillei* are two examples of antibiotic-producing alkaliphiles (22). In a recent study by Henciya et al. (2020), the antibacterial activity of halophilic bacteria against drug-resistant microbes was remarkable (25). Members of the *Thermoactinomycetaceae* family are among the extremophilic microbial sources that are poorly studied. Several studies have shown that they produce many bioactive compounds with various therapeutic activities such as antimicrobial agents (6). In this study, we have shown that *Laceyella sacchari* UTMC 2705, *Thermoactinomyces* sp. UTMC 2721 and *Laceyella* sp. UTMC 2731 have efficient anti-MRSA activity. The presence of uv-active compounds in HPLC analysis is further evidence of the bioactivity of FBEs (26). Short seeding and fermentation periods and good enzymatic activities of these active strains (Table 4) can be considered as remarkable advantages to use them for the production of low- cost drugs in biotechnology companies.

According to Bergey's Manual of Systematics of Archaea and Bacteria (2011), *Laceyella sacchari* and *Thermoactinomyces daqus* belong to the family *Thermoactinomycetaceae*.

The production of bioactive compounds from members of the *Thermoactinomycetaceae* family has been reported previously. Thermorubin, an orange-red pigment, from *Thermoactinomyces* antibioticus was the first isolated biocompound with antibiotic activity from a member of this family (22). According to Bulkley et al. thermorubin stops translation in the initiation phase by binding to inter-subunit bridge B2a of the ribosome (27). Thermoactinoamide A, with cyclic hexapeptide structure, is also a recently described antibiotic from *Thermoactinomyces vulgaris* (28).

According to Dammak et al. based on the HRESI-MS spectral data, the cyclic lipopeptide Gramicidin S and four cyclic dipeptides were detected in the fermentation broth of *Paludifilum halophilum*, a new genus of the *Thermoactinomycetaceae* family isolated from the surface sediment of a sun saline with antimicrobial activity against *S. aureus* and other pathogenic bacteria (29). Later, in 2020, Dammak et al. discovered N-(1-carboxy-ethyl)-phthalamic Acid as the first secondary metabolite from *Paludifilum halophilum* with antibacterial activity (30).

Mechercharimyces is another member of the *Thermoactinomycetaceae* that produces two antitumor agents (Mechercharmycin A and Urukthapelstatin A) (8). *Thermoactinomyces daqus* was first isolated in 2014 from a high-temperature sample of daqu (a traditional beverage) collected during the production of sesame-flavored liquor in China. Most studies focus on its role in Chinese liquor production and qual*ity improvement (31).* Whole genome sequencing of *Thermoactinomyces daqus* H-18 was performed in 2015 (32). However, there is no report on its antimicrobial activity yet. *Laceyella sacchari* was first isolated from bagasse in 1953. Its anti-MRSA activity has already been qualitatively reported by agar overlay method (33). The herbicidal activity of its metabolites was also reported in 2014 (34).

In conlusion, Extremophilic microorganisms are promising sources of bioactive compounds. The results of this study suggest that the ability of members of the *Thermoactinomycetaceae* family to produce potent, heat-stable, and substantial antimicrobial compounds may be considered a promising source of antimicrobial agents for future investigation.

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