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Original Article

Characterization of six bioactive compounds from two soil-derived *Streptomyces* strains, F9 and F4 from Iran

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Abstract

Background and purpose: Actinobacteria are highly valuable in the pharmaceutical industry due to their unlimited capacity to produce natural products. In line with the screening of actinomycetes to discover new antibacterial agents, this study isolated and elucidated bioactive compounds from two *Streptomyces* strains, F9 (99.88% similarity with *S. chryseus*) and F4 (99.14% similarity with *S. rectiviolaceus*).
Experimental approach: The present study followed a rigorous experimental approach. Previously, two *Streptomyces* strains, F9 and F4, were isolated and identified. These strains were then selected for the isolation and elucidation of secondary metabolites. The crude extract was semi-purified using Waters, and the active fractions were further purified using Agilent. The structure of the isolated compounds was elucidated through detailed spectroscopic analysis to ensure the accuracy and reliability of the findings.
Findings/Results: Four fractions isolated from *strain F9* showed antibacterial activity against test microorganisms. HR-ESI-MS, fragmentation pattern, and database search identified chrysomycins B and C. The structure of chrysomycins A and 4'-O-acetylated A was confirmed by 1D and 2D-NMR data. In addition, the findings characterized 2 pigments produced by strain F4 using HR-ESI-MS and fragmentation pattern (ESI-MS/MS), which revealed that major and minor peaks corresponded to butyl-meta-cycloheptylprodiginine and undecylprodiginine, respectively.
Conclusion and implications: Six bioactive compounds, including 4 chrysomycins and 2 pigments, were isolated from 2 *Streptomyces* strains, F9 and F4. Importantly, this was the first report on isolating chrysomycins A-C and 4'-*O*-acetylated A from *S. chryseus*.

Keywords: Anti-bacterial agents; Butyl-meta-cycloheptylprodiginine; Chrysomycin; *Streptomyces*; Undecylprodiginine.

INTRODUCTION

Antibiotics have revolutionized the treatment of common bacterial infections, have played a crucial role in reducing mortality (1). Historically, nature is a rich source of therapeutic agents (2). Actinobacteria include a significant number of the bacterial population in most soil types. They are very effective in the pharmaceutical industry due to their unlimited capacity to produce natural products with a high chemical diversity and a broad spectrum of biological activities. Almost 50% of Actinobacteria belong to the genus *Streptomyces*, and 75% of commercially essential antibiotics are derived from this genus (3). Among them, polyketides are a significant source of novel chemotherapeutic agents (4).

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Napthocoumarin-linked to C-glycoside antibiotics are divided into 3 main classes based on the sugar type containing 1. gilvocarcin (furanose), 2. chrysomycin and polycarcin (pyranose), and 3. ravidomycin (pyranosamine); however, in all classes, the basic naphthocoumarin skeleton is fixed. The structural analysis of the antibiotics indicated 5 different substitution types reported at the C-8 position (methyl, vinyl, ethyl, epoxide, and hydroxyl-containing moieties). Significantly, the functionalities of methyl, vinyl, and ethyl are repeatedly reported (2,5). Chrysomycins are potent antibacterial (effective against gram-positive bacteria) and antitumor antibiotics. Chrysomycin, a yellow crystalline antibiotic, was first isolated from an unidentified *Streptomyces* by Strelitz *et al*. in 1955 (6). Efforts to isolate and determine the structure of chrysomycins A and B continued until 1982 (7). In 2013, chrysomycin C, as the analog of chrysomycin A (vinyl was replaced with ethyl), and 2 other analogs, chrysomycins D and E, were reported by Jain *et al*. (2). Recently, Wada *et al*. isolated 4'-*O*-acetylchrysomycin A and 4'-*O*-acetylchrysomycin B from *Streptomyces* sp. strain MG271-CF2 with 100-fold more substantial toxicity in some cancer cells compared with normal cells (5). Studies have shown that while acetylation increases the cytotoxicity of chrysomycin B, it reduces the activity of chrysomycin A (8,9). One of the most prevalent and serious infectious diseases is tuberculosis, caused mostly by *Mycobacterium tuberculosis*. Since there is still no great advance to fight this disease, a serious need exists to produce new antimycobacterial drugs with enhanced features such as increased activity against multidrug resistance with less toxicity (10). The anti-bacterial effect of chrysomycin A against *M. tuberculosis* and multidrug-resistant *tuberculosis* was shown in previous studies (8,9).

Prodiginine analogs are a family of red pigments that consist of a tripyrrole (pyrrolydipyrrolylmethene) backbone and a methoxy function on the B-ring. Recently, prodiginines have received more attention due to their proapoptotic potential as anticancer agents. Prodiginines have anticancer activity against many cell lines, including breast, colon, lung, and kidney, with low cytotoxicity against non-cancerous cells (11,12). Niakani *et al*. showed that prodigiosin, as an anti-proliferative natural substance, induces apoptosis in K562 cancer cells through caspase-3 activation, resulting in DNA fragmentation (13).

Prodigiosin is a secondary microbial metabolite colored red due to the highly conjugated planar pyrrolylpyrromethene chromophore. The prodigiosin pyrrolylpyrromethene skeleton consists of a common bipyrrole in which 2 pyrrole rings, A and the methyoxypyrrole ring B, are directly linked, and a monopyrrole unit (ring C) is joined *via* a methene bridge. The red-colored pigments with this tripyrrole aromatic moiety are known as the members of the prodiginine family (14). The prodiginine family includes prodigiosin, undecylprodiginine (prodigiosin 25C) and its carbocyclic derivatives such as ethyl-meta-cyclononylprodiginine (metacycloprodigiosin; streptorubin A), butyl-meta-cycloheptylprodiginine (streptorubin B), methylcyclodecylprodiginine (12), prodigiosin 25B (15), prodigiosin R1, roseophilin, etc. The carbocyclic derivatives of undecylprodiginine, particularly metacycloprodigiosin, are more potent than undecylprodiginine itself (12). It was possible to readily identify all the prodiginine analogs in the positive ion mode and achieve necessary and sufficient structural information without the need for further purification, especially if there was a low abundance of prodiginine analogs in a complex mixture due to available basic nitrogen atoms and similarities in the fragmentation patterns (15,16). It is a considerable advantage because purification is often complicated due to their similar physical and chemical properties (17). A stable odd-electron cation (OE^+) fragment ion is initially formed by losing a 15 Da neutral fragment, a methyl radical. In a competitive pathway, an even-electron (EE^+) fragment is derived from the loss of methanol, but the abundance of EE^+ fragments is substantially lower than OE^+ fragments (15).

Two *Streptomyces* strains, F9 and F4, were isolated previously (18) and selected for isolation and elucidation of secondary metabolites in this study. Strain F9 (99.88% similarity with *S. chryseus* and GenBank

accession number KX417085) was active against 2 ATCC strains, including *Staphylococcus aureus* and *Bacillus subtilis,* and 2 hospital-acquired strains, including methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant *Enterococcus* (VRE). Strain F4 (99.14% similarity with *S. rectiviolaceus* and GenBank accession number KX229768) was active against *S. aureus*, *B. subtilis,* MRSA, *Rhizopus* sp., and *Candida albicans* (19).

Herein, this study discussed the isolation and characterization of 4 chrysomycins B (compound 1), A (compound 2), C (compound 3), and 4'-*O*-acetylated A (compound 4) produced by strain F9 and 2 prodigiosin-like pigments, butyl-meta-cycloheptylprodiginine and undecylprodiginine, from strain F4 isolated from the soil sample collected from Fereydunshahr, Isfahan, Iran.

Antimicrobial resistance and the emergence of multidrug-resistant bacteria are a global health threat facing the human population (20), with potential annual deaths projected to reach 10 million by 2050. Vancomycin, a last-line antibacterial agent for MRSA infections, is also facing challenges with the emergence of vancomycin-resistant *S. aureus* and vancomycin-intermediate *S. aureus* cases. In this context, the urgent need for novel antibiotics with new mechanisms of action to combat antimicrobial resistance is evident. Chrysomycin A, identified as a drug lead, demonstrated a potent bactericidal effect on MRSA by targeting multiple critical cellular processes. This potential made it a promising candidate for therapeutic application (21).

MATERIALS AND METHODS

Primary evaluation of antibiotic production

Antibiotic production was evaluated in 3 different media, including 1. starch casein (SC) containing 10 g/L soluble starch, 0.3 g/L casein, 2 g/L K_2HPO_4, 2 g/L KNO_3, 2 g/L NaCl, 0.05 g/L $MgSO_4.7H_2O$, 0.02 g/L $CaCO_3$, and 0.01 g/L $FeSO_4.7H_2O$ (15 g/L agar added for solid medium) (22,23); 2. SM containing 20 g/L mannitol and 20 g/L without oil soy flour (24); 3. M2 containing 10 g/L malt extract, 10 g/L mannitol, 4 g/L yeast extract, and 4 g/L glucose. pH was adjusted to 7.2 with HCl 37% and 1 M NaOH.

Fermentation and preparation of crude extract

Inoculation was aseptically done from a one-week-old culture in 100 mL flasks, each containing 10 mL of SC medium, and incubated on a rotary shaker at 120 rpm and 30 °C for 2 days. Five-litre flasks containing 1 L of SC medium were inoculated with 1% seed and incubated on a rotary shaker at 120 rpm and 30 °C for 10 days. After 10 days, the cells from the liquid culture were removed by centrifugation at 4000 rpm for 15 min. The obtained supernatant was filtered through filter paper. 2% XAD-16 macroporous adsorption resin was added to the filtered supernatant and then shaken at 4 °C for 1 h. The resins were collected on a filter. The adsorbed material was eluted with methanol (twice, 100 mL each time) and then stirred for 1 h. An equal volume of ethyl acetate was added to the culture supernatant, shaken for 1 h, and then set statically for 30 min. The solvent phase was separated from the aqueous phase using a separating funnel. The solvent phase (methanol and ethyl acetate) was concentrated in a rotary vacuum evaporator and a water bath at 30-40 °C to give a crude extract (1 g). The dried crude extract was stored in sterile capped bottles and refrigerated at 4 °C until required.

Analysis procedures

Liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS) analysis was carried out on an Impact II mass spectrometer (Bruker, Germany) using an Acquity UPLC BEH C18 column, 130 Å, 1.7 µm, and 2.1 mm × 50 mm (Waters, USA) with an Acquity UPLC BEH C18 pre-column, 130 Å, 1.7 µm, and 2.1 mm × 5 mm (Waters, USA). The acetonitrile/H2O (ACN/H_2O) (supplemented with 0.1% formic acid) gradient from 5% to 95% was used at a flow rate of 0.4 mL/min and temperature of 40 °C (12 min), demonstrating the precision and control in our methodology. This was monitored in the wavelength range of 200-600 nm. Ultraviolet-visible (UV-Vis) spectra were recorded on UV/Vis-Detector 200-600 nm (Thermo Fisher,

USA). The reaction mixture was purified by preparative HPLC (Waters and Agilent, USA). Semi-purification was carried out on Waters system using an XBridge Peptide BEH C18 OBD Prep Column, 300 Å, 5 µm, 19 mm × 150 mm with 38-53% (for strain F9) and 5-95% (for strain F4) ACN/H_2O (supplemented with 0.1% FA) gradient at a flow rate of 24 mL/min and temperature 40 °C (15 min) and monitored in the wavelength range of 200-600 nm. The final purification of active fractions isolated from the F9 strain crude extract was carried out on Agilent using an Eclipse XDB C18 column, 5 µm, 9.5 mm × 250 mm with 45-80% ACN/H_2O (supplemented with 0.1% FA) gradient at a flow rate of 3 mL/min and monitored at a wavelength 248 nm.

^1H, ^{13}C nuclear magnetic resonance (NMR) and 2D-NMR spectra were recorded on a 600 MHz spectrometer (Bruker, Germany). Chemical data were recorded in parts per million (ppm, δ values) downfield from tetramethylsilane and referenced to the residual protons or carbons of the NMR solvent (dimethyl sulfoxide (DMSO), 2.5 ppm for proton and 39.5 ppm for carbon). The isolated compounds were confirmed by high-resolution electrospray ionization mass spectrometry (HR-ESI-MS) and NMR data.

Supplementary materials

The supplementary materials for this article can be found online at: https://github.com/etemadifar689/Supplementary-Article-RPS-254-23/blob/main/Supplementary%20RPS-254-23.pdf

RESULTS

Screening of the best strains

Two strains (F4 and F9) grown on SCA medium showed high antimicrobial effects against MRSA, VRE, *Rhizopus,* and *Candida* strains (Figs. S1 and S2) in the initial screening.

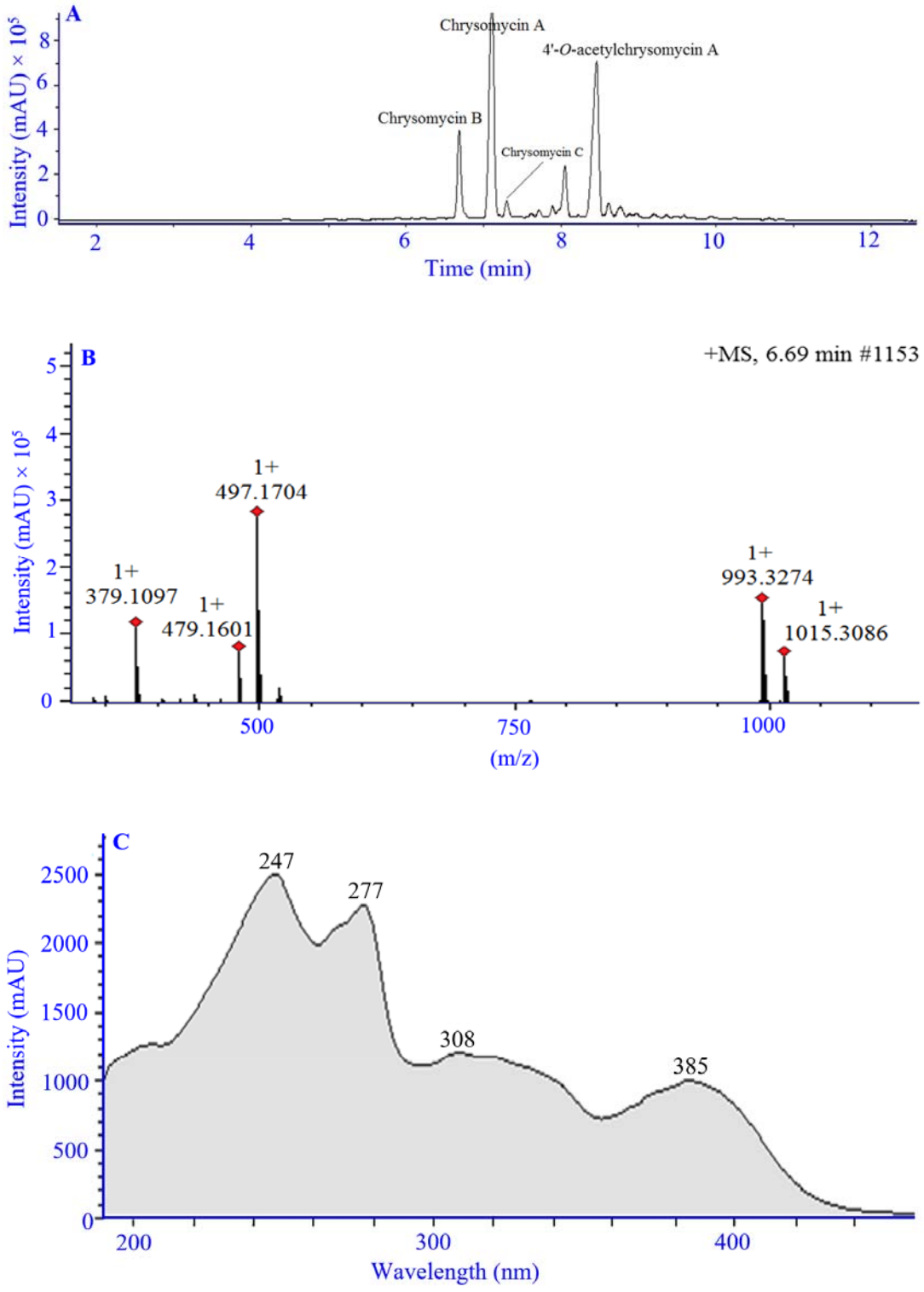
Evaluation of 3 different media for the production of antibiotics

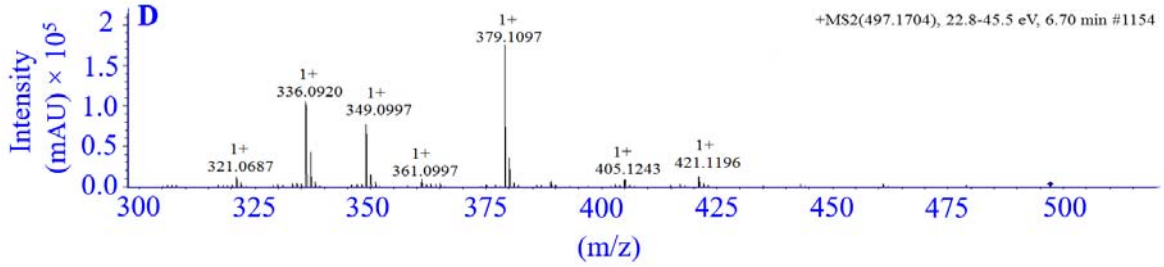
None of the strains (F4 and F9) could grow in the M2 medium. They had fast growth rates on SM medium, but fewer antibiotics were favored. Despite the low growth rate, the SC medium supported the highest antibiotic level in strains F4 and F9 and was selected for large-scale production (Fig. S3).

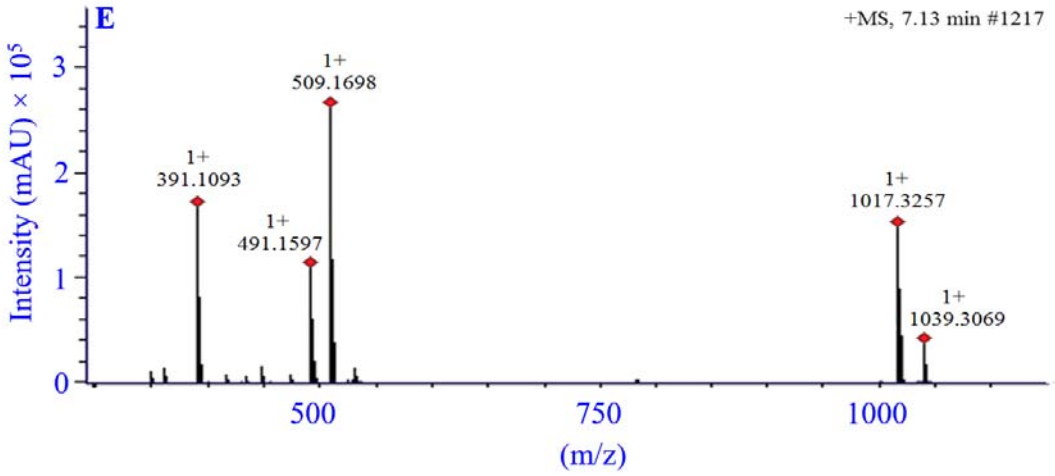
Fractionation and purification of strain F9 crude extract

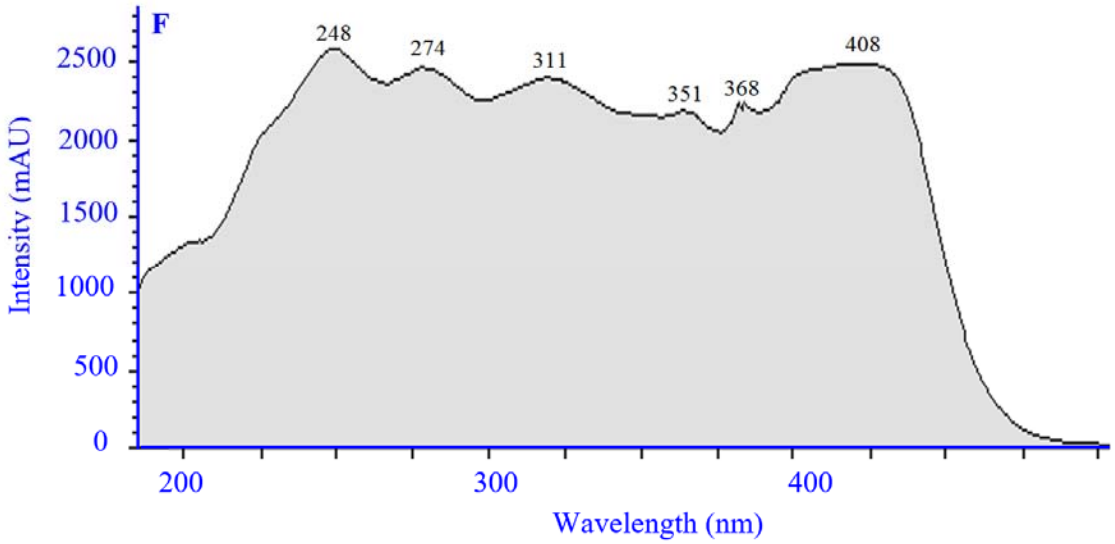
The crude extract was dissolved in DMSO/methanol/isopropanol (7/2/1) and then subjected to Waters preparative HPLC to give 10 fractions (fractions 1-10). Fractions 4, 5, 6, and 7 had antibacterial activity. They were further purified by Agilent semipreparative HPLC and profiled by using liquid chromatography-mass spectrometry (LC-MS). The HPLC chromatogram (Fig. 1A) displayed 4 major peaks. The first peak was at 6.69 min and its HR-ESI-MS at *m/z* 497.1704 $[M + H]^+$ (calculated for $C_{27}H_{29}O_9$), the second pick at 7.14 min, HR-ESI-MS at *m/z* 509.1698 $[M + H]^+$ (calculated for $C_{28}H_{29}O_9$), the third pick at 7.29 min and its HR-ESI-MS at *m/z* 511.1854 $[M + H]^+$ (calculated for $C_{28}H_{31}O_9$), and the last pick was at 8.47 min and its HR-ESI-MS at *m/z* 551.1796 $[M + H]^+$ (calculated for $C_{30}H_{31}O_{10}$), all with similar HR-ESI-MS (positive mode) (Fig. 1B, E, H, and K), UV (Fig. 1C, F, I, and L), and fragmentation (Fig. 1D, G, J, and M) pattern, denoting that all 4 compounds belonged to the same chemical group.

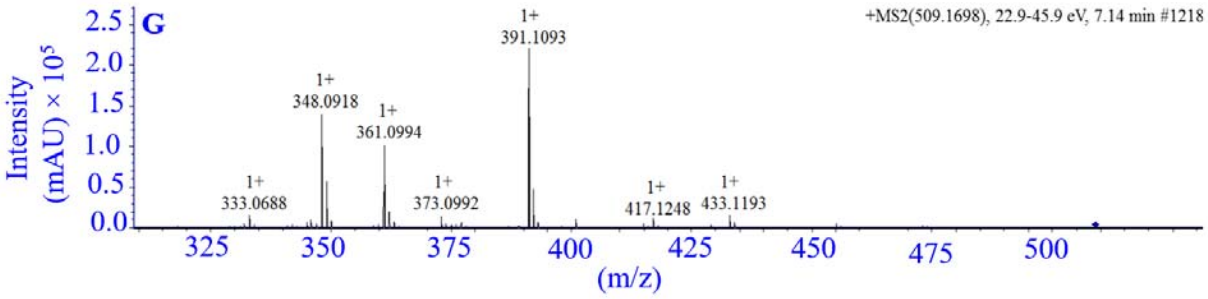
The mass, molecular formula, and UV spectra of 4 compounds were used as input data in Scifinder, Dictionary of Natural Products, and Streptome DB Databases. The obtained information indicated the presence of 3 known chrysomycins, including B (compound 1), A (compound 2), and C (compound 3), at t_R 6.69, 7.14, and 7.29 min, respectively. The mass and UV data of the major peak of compound 4, eluted at 8.47 min, were found in no database, but its structure was elucidated by Wada *et al*. (5), which led to its identification as 4'-*O*-acetylchrysomycin A (compound 4). This study could isolate 2 major compounds, 2 and 4, in quantities sufficient for NMR spectral characterization. They were confirmed as chrysomycin A and 4'-*O*-acetylchrysomycin A by analyzing their 1D and 2D NMR (Table 1, Fig. 2, and Fig. S4) and HR-ESI-MS data.

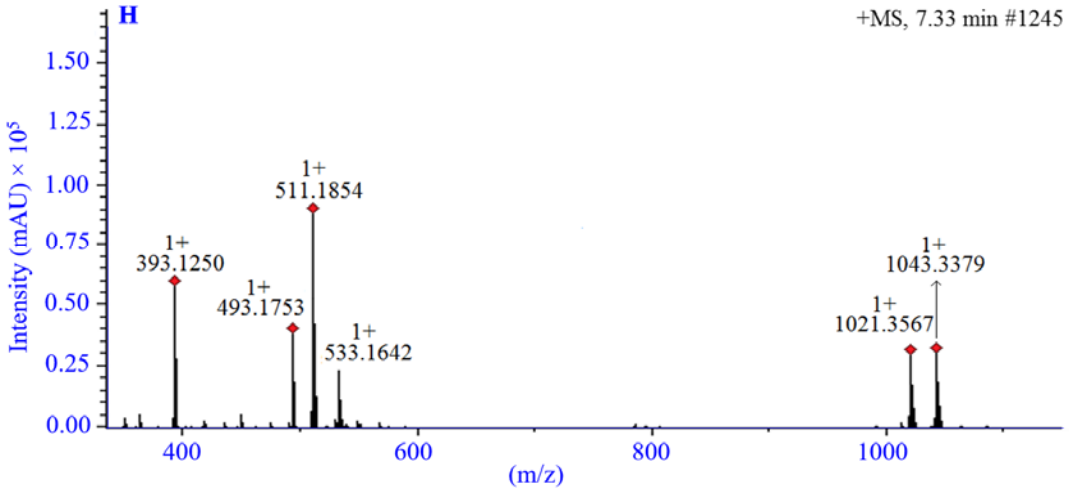

A
Chrysomycin A
4'-O-acetylchrysomycin A
Chrysomycin B
Chrysomycin C
Intensity (mAU) × 10^5
Time (min)
B
+MS, 6.69 min #1153
1+
497.1704
1+
379.1097
1+
479.1601
1+
993.3274
1+
1015.3086
Intensity (mAU) × 10^5
(m/z)
C
247
277
308
385
Intensity (mAU)
Wavelength (nm)

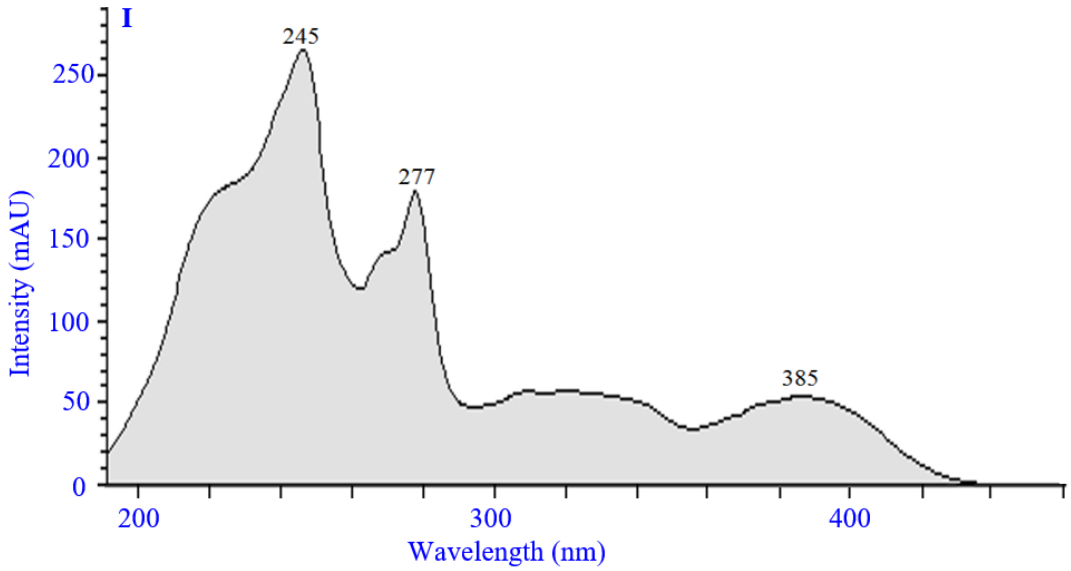

D
+MS2(497.1704), 22.8-45.5 eV, 6.70 min #1154
Intensity (mAU) × 10^5
1+ 379.1097
1+ 336.0920
1+ 349.0997
1+ 321.0687
1+ 361.0997
1+ 405.1243
1+ 421.1196
(m/z)

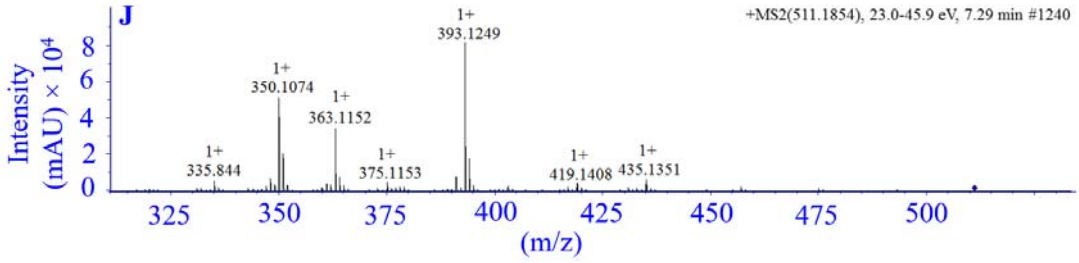

E
+MS, 7.13 min #1217
Intensity (mAU) × 10^5
1+ 509.1698
1+ 391.1093
1+ 491.1597
1+ 1017.3257
1+ 1039.3069
(m/z)

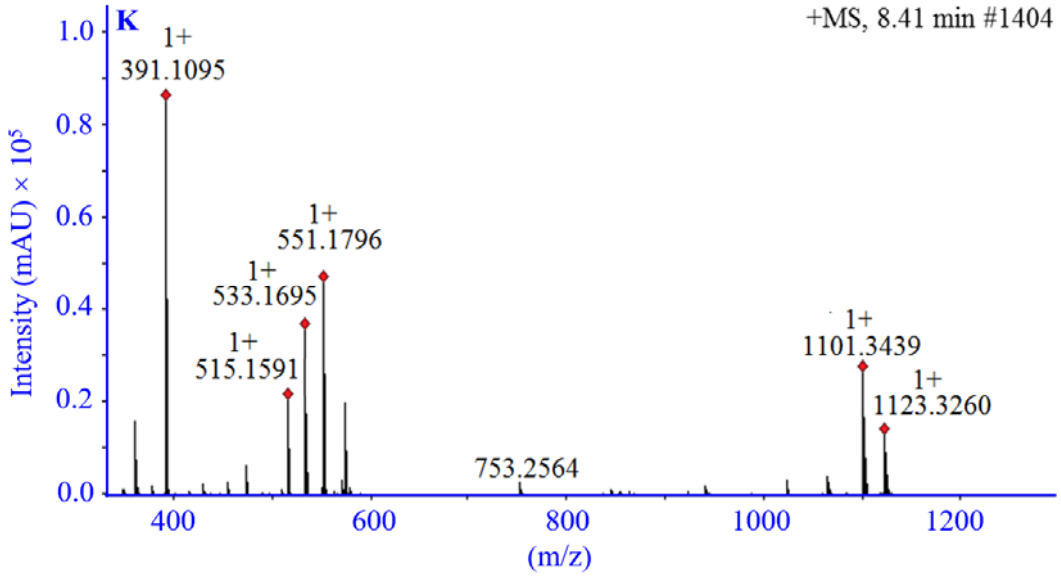

F
Intensity (mAU)
248
274
311
351
368
408
Wavelength (nm)

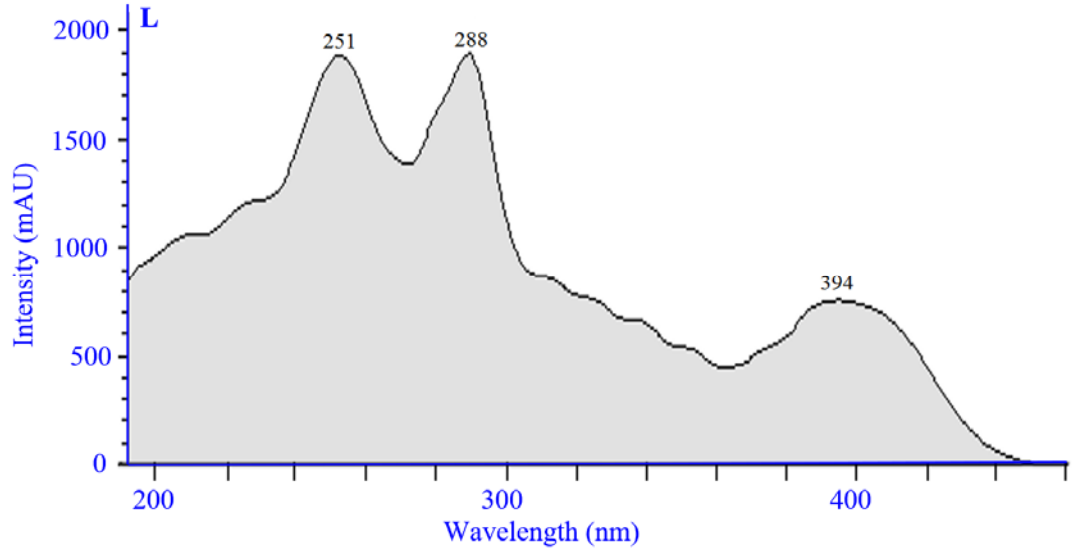

G
+MS2(509.1698), 22.9-45.9 eV, 7.14 min #1218
Intensity (mAU) × 10⁵
1+ 391.1093
1+ 348.0918
1+ 361.0994
1+ 333.0688
1+ 373.0992
1+ 417.1248
1+ 433.1193
(m/z)


H
+MS, 7.33 min #1245
Intensity (mAU) × 10⁵
1+ 511.1854
1+ 393.1250
1+ 493.1753
1+ 533.1642
1+ 1043.3379
1+ 1021.3567
(m/z)


I
245
277
385
Intensity (mAU)
Wavelength (nm)


J
+MS2(511.1854), 23.0-45.9 eV, 7.29 min #1240
Intensity (mAU) × 10⁴
1+ 393.1249
1+ 350.1074
1+ 363.1152
1+ 335.844
1+ 375.1153
1+ 419.1408
1+ 435.1351
(m/z)


K
+MS, 8.41 min #1404
Intensity (mAU) × 10⁵
1+ 391.1095
1+ 551.1796
1+ 533.1695
1+ 515.1591
1+ 1101.3439
1+ 1123.3260
753.2564
(m/z)


L
Intensity (mAU)
251
288
394
Wavelength (nm)

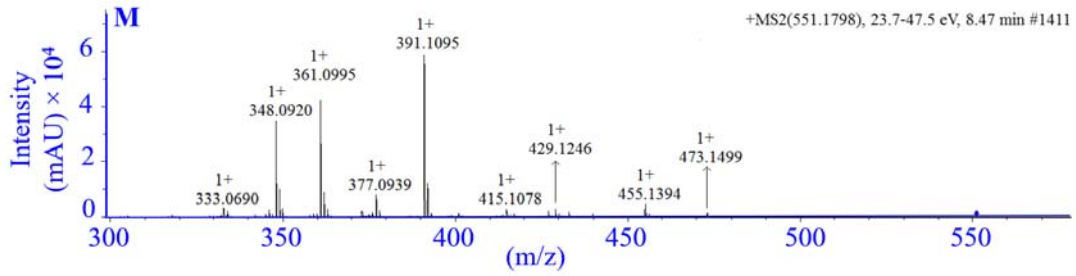


Fig. 1. HR-ESI-MS spectrum of crude mixture at λ_{max} 248 nm showing (A) identification of 4 known chrysomycins B, A, C and 4'-*O*-acetylated A; HR-ESI-MS (positive mode), UV spectra, and fragmentation pattern for peak at t_R (B, C, D), 6.69 min; (E, F, G), 7.14 min; (H, I, J), 7.29 min; (K, L, M), 8.47 min. HR-ESI-MS, High-resolution electrospray ionization mass spectrometry.

Table 1. The ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR spectroscopic data for compounds 2 and 4 (DMSO-d_6).

Position	Compound 2		Compound 4	
	δ_C	$J\delta_H$ (mult., *J* in Hz)	δ_C	$J\delta_H$ (mult., *J* in Hz)
1	153.77		154.1, C	
2	112.57	6.98 (d, 8.4)	112.6, CH	6.99 (d, 8.4)
3	129.3	7.84 (d, 8.4)	129.6, CH	7.77 (d, 8.4)
4	128.4		127.5, C	
4a	125.4		129.8, C	
4b	142.91		143.0, C	
6	160.21		160.5, C	
6a	122.5		123.5, C	
7	121.54	8.01 (s)	119.7, CH	8.03 (br s)
8	139.17		139.9, C	
9	114.63	7.75 (s)	115.5, CH	7.78 (br s)
10	157.85		158.0, C	
10a	123.18		123.8, C	
10b	115.54		115.8, C	
11	100.82	8.48 (s)	102.3, CH	8.53 (s)
12	152.25		152.6, C	
12a	115.54		115.5, C	
1'	75.11	6.02 (d, 9.5)	75.2, CH	6.04 (d, 9.5)
2'	73.49	3.68 (t, 9)	72.6, CH	3.70 (d, 7.3)
3'	72.36		72.0, C	
4'	76.13	3.14 (d, 7.7)	77.6, CH	4.76 (br s)
5'	71.14	4.52 (q, 6.6)	69.8, CH	4.72 (q, 7)
6'	16.64	1.02 (d, 6.2)	17.4, CH_3	0.88 (d, 6.5)
7'	24.29	1.26 (s)	23.4, CH_3	1.17 (s)
10-OMe	57.07	4.17 (s)	57.3, CH_3	4.19 (s)
12-OMe	56.69	4.12 (s)	56.8, CH_3	4.14 (s)
-CH =	135.51	6.96 (dd, 7, 18)	135.7, CH	6.96 (dd, 11, 18)
= CH_2	117.11	5.51 (d, 11), 6.15 (d, 17.6)	117.9, CH_2	5.52 (d, 11), 6.17 (d, 18)
-$\underline{C}OCH_3$			170.7, C	
-CO$\underline{CH_3}$			21.2, CH_3	2.12 (s)
1-OH		9.82 (s)		
2'-OH		4.19 (br s)		
3'-OH		4.20 (s)		
4'-OH		4.59 (d, 7.7)		

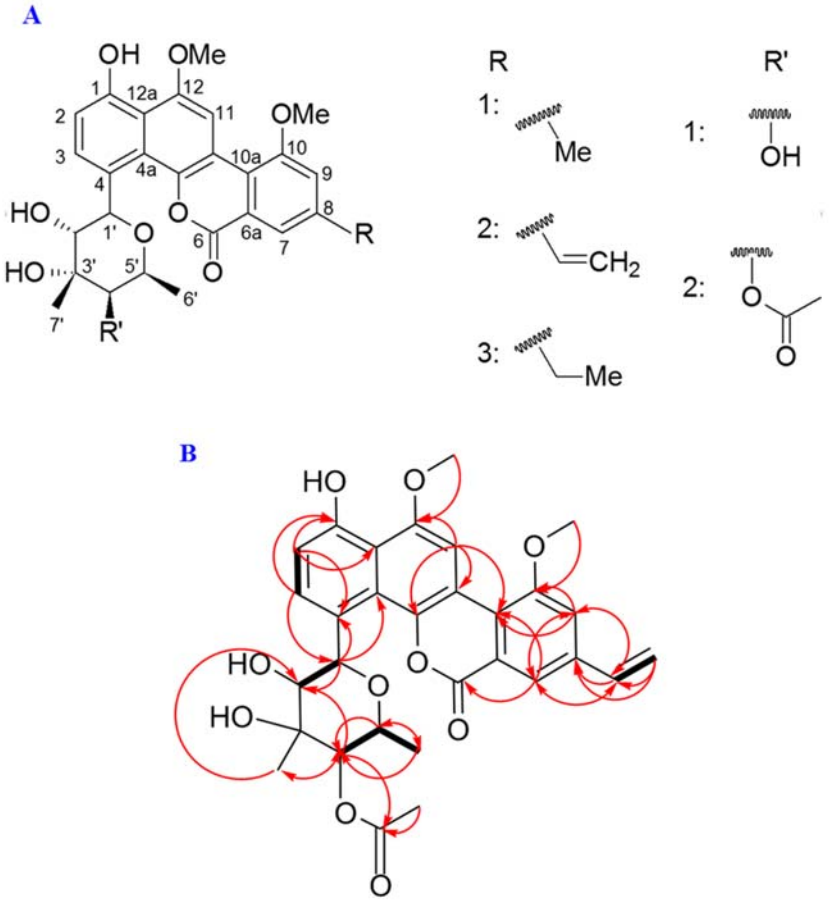


Fig. 2. Chemical structures of (A) chrysomycins A-C and 4'-*O*-acetylated A and (B) HMBC (H→C) and ^{1}H–^{1}H COSY (—) correlations.

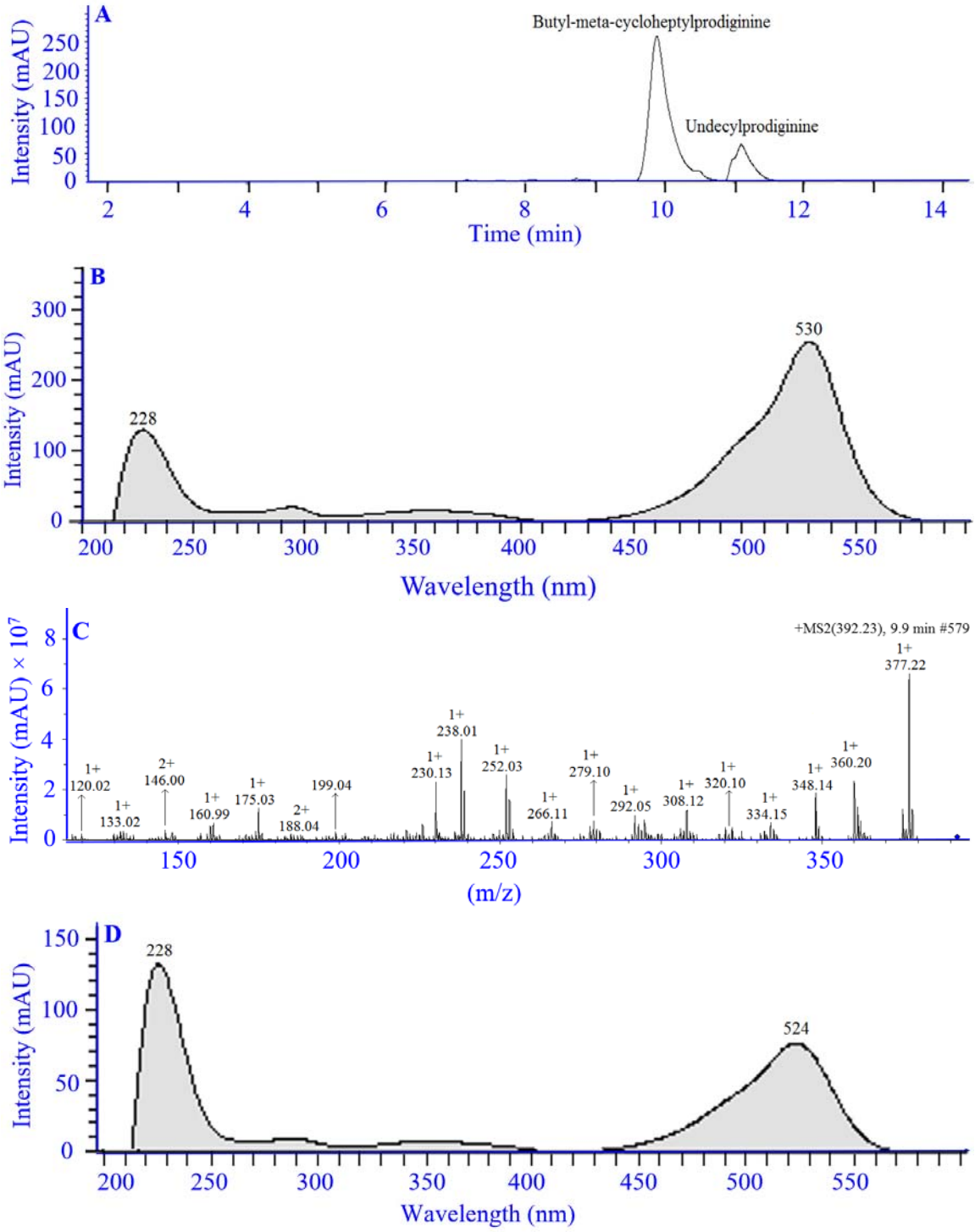
Fractionation and purification of strain F4 crude extract

The semi-purified strain F4 crude extract was prepared using preparative HPLC Waters. Fractions 9 (major peak) and 10 (minor peak) with t_R 9.9 and 11.2 min (Fig. 3A) had antibacterial activity against test microorganisms.

The HR-ESI-MS analysis (Fig. 3A), an exact method, revealed parent ions of *m*/*z* 392.2694 $[M + H]^+$ (calculated for $C_{25}H_{33}N_3O$) and *m*/*z* 394.2847 $[M + H]^+$ (calculated for $C_{25}H_{35}N_3O$) with λ_{max} at 530 and 524 nm in methanol solution, respectively (Fig. 3B and D). Both compounds were dark red-purple, confirming the accuracy of the current analysis.

The molecular formula of 2 compounds was used as input data in different databases. A list of compounds matching $C_{25}H_{33}N_3O$ consisted of ethyl-meta-cyclononylprodiginine (metacycloprodigiosin, streptorubin A), butyl-meta-cycloheptylprodiginine (streptorubin B), methylcyclodecylprodiginine, and prodigiosin 25B (Fig. 4). The oxidative cyclization of undecylprodiginine between C-4 of ring C and C-7', C-9', or C-10' of the hydrocarbon chain gave butyl-meta-cycloheptylprodiginine, ethyl-meta-cyclononylprodiginine, and methylcyclodecylprodiginine, respectively. The ESI-MS/MS technique (Fig. 3C) confirmed the presence of butyl-meta-cycloheptylprodiginine as the major component of the mixture.

The fragmentation pattern of the minor component, combined with the molecular weight (Fig. 3E) and UV-Vis absorbance (Fig. 3D) of this compound, suggested it as undecylprodiginine.


A
Butyl-meta-cycloheptylprodiginine
Undecylprodiginine
Intensity (mAU)
Time (min)
B
228
530
Intensity (mAU)
Wavelength (nm)
C
+MS2(392.23), 9.9 min #579
Intensity (mAU) × 10^7
(m/z)
D
228
524
Intensity (mAU)
Wavelength (nm)

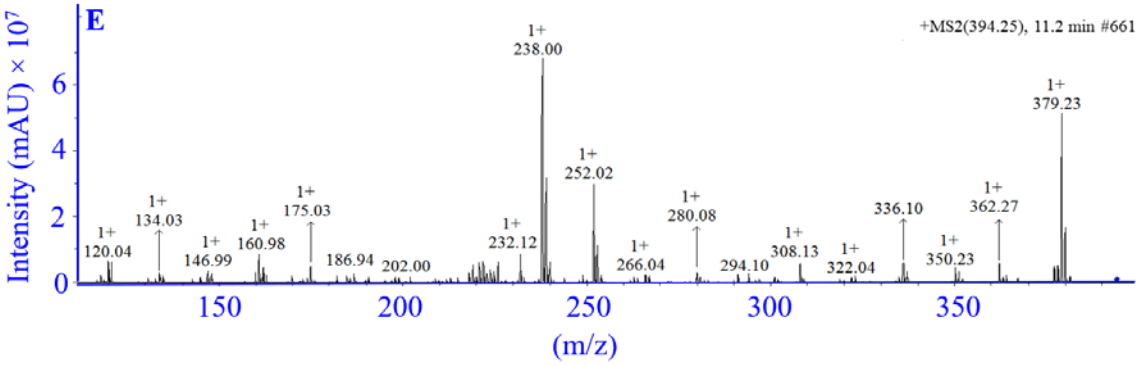


Fig. 3. HR-ESI-MS spectrum of crude mixture at λ_{max} 530 nm showing (A) identification of 2 known butyl-meta-cycloheptylprodiginine and undecylprodiginine; UV and MS/MS spectra of peaks at t_R (B and C, respectively) 9.9 min; (D and E, respectively) 11.2 min. HR-ESI-MS, High-resolution electrospray ionization mass spectrometry; MS, mass spectrometry.

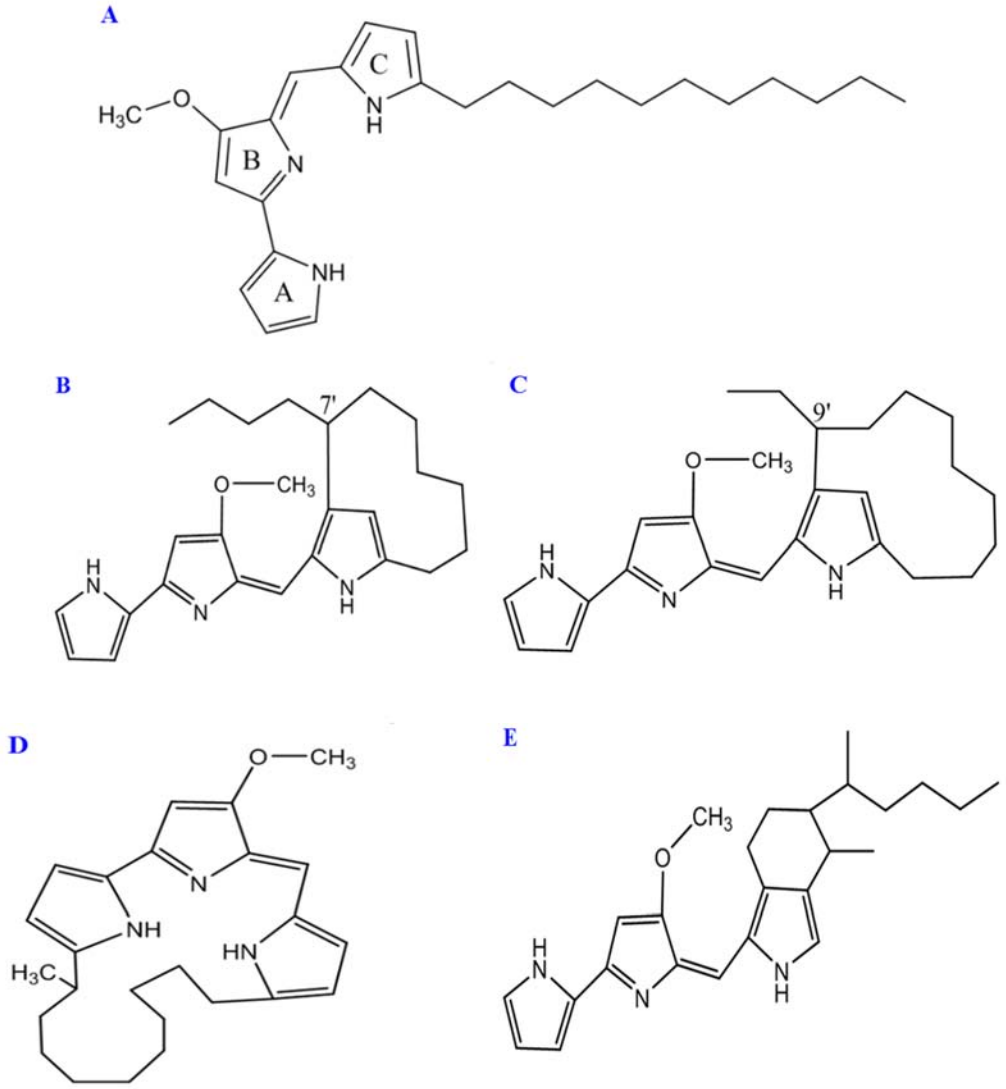


Fig. 4. The structure of undecylprodiginine and its cyclic derivatives. (A) Undecylprodigiosin; (B) butyl-meta-cycloheptylprodigionine; (C) ethyl-meta-cyclononylprodiginine; (D) methyl-cyclodecylprodiginine; and (E) prodigiosin 25B.

DISCUSSION

F9 strain

The ^{1}H NMR spectrum of compound 2 with the molecular formula of $C_{28}H_{29}O_9$ from the HR-ESI-MS peak at *m/z* 509.1698 $[M + H]^+$ showed 20 proton signals, including 5 aromatic protons at δ_H 8.53 ~ 6.99, 3 vinyl protons, 2 methoxy groups (6 protons), 4 carbohydrate ring protons, and 2 methyl groups (6 protons) at δ_H 6.96 ~ 1.02 and 4 oxygenated protons, which did not exhibit HSQC correlations at δ_H 9.82 (indicating the presence of phenolic group) and 4.59 ~ 4.19 (indicating the presence of a carbohydrate ring). The ^{13}C NMR spectrum displayed 28 carbon signals that were classified by HSQC and HMBC spectra, including 17 aromatic carbons (5 olefinic methine carbons, 4 oxygenated and 1 conjugated ester carbonyl carbon) and 2 vinyl carbons attached to the aromatic ring at δ_C 157.85 ~ 100.82, 5 pyranosyl ring carbons at δ_C 76.13 ~ 71.14, 2 methoxy carbons at δ_C 57.07 and 56.69 and 2 methyl carbons at δ_C 24.29 and 16.64. From COSY and HMBC correlations (Fig. 2), along with the values of coupling constants (Table 1), the carbohydrate ring was identified and confirmed as a pyranoside. The ^{1}H-^{1}H COSY correlation between H-1' (δ_H 6.02) and H-2' (δ_H 3.68), along with the HMBC correlation of H-1' (δ_H 6.02) to C-4 (δ_C 128.4) and C-4a (δ_C 125.4) and H-3 (δ_H 7.84) to C-1' (δ_C 75.11) showed the connectivity between the sugar ring with the aromatic group. The detailed analysis of HR-ESI-MS and NMR data and the reassuring comparison to literature (2,7) indicated that the isolated compound 2 was indeed chrysomycin A.

The mass of the compound 4 (*m/z* 551 $[M + H]^+$) was increased by 42 compared to the compound 2 (*m/z* 497 $[M + H]^+$). This intriguing observation suggested that there is probably an *O*-acetyl group in compound 4. The ^{13}C NMR and ^{1}H NMR spectra resembled those of chrysomycin A, except for the appearance of an acetyl group (δ_C 170.7 and 21.2; δ_H 2.12). In the HMBC spectrum of compound 4, correlations from the methyl proton (δ_H 2.12) to the carboxyl carbon at δ_C 170.7, from H-4' (δ_H 4.76) to-$\underline{C}OCH_3$ (δ_C 170.7)/C-7' (δ_C 23.4)/C-2' (δ_C 72.6), from H-5' (δ_H 4.72) to C-4' (δ_C 77.6)/C-6' (δ_C 17.4), from H-6' (δ_H 0.88) to C-5' (δ_C 69.8)/C-4', from H-7' (δ_H 1.17) to C-4'/C-2' and from H-1' (δ_H 6.04) to C-2'/C-4 (δ_C 127.5)/C-4a (δ_C 129.8) demonstrated that the compound 4 was the analog of chrysomycin A in which 4'-OH was acetylated. Thus, structure 4 was established as 4'-*O*-acetylchrysomycin A (Table 1 and Fig. 2). The supporting information for elucidating components 2 and 4 was presented in Fig. S4.

The poor production of components 1 and 3 did not allow for the larger-scale isolation necessary for NMR analysis. HR-ESI-MS analyses showed that the mass of the compound 3 (*m/z* 511 $[M + H]^+$) was increased by 14 compared to the compound 1 (*m/z* 497 $[M + H]^+$) and 2 compared to the compound 2 (*m/z* 509 $[M + H]^+$). We assumed that compounds 1 and 3 were formed by reducing the vinyl moiety of chrysomycin A at C-8 to methyl and ethyl moieties, respectively.

Ni *et al*. showed that the optimal medium for the production of chrysomycin A by *Streptomyces* sp. 891 consisted of glucose, corn starch, soybean flour, and $CaCO_3$ (25). The present study also supported the highest antibiotic level in the SC medium (containing starch, casein, and $CaCO_3$).

F4 strain

The ESI-MS/MS technique was used to characterize major pigment with the molecular formula of $C_{25}H_{33}N_3O$ (Fig. 3C). As Chen *et al*. (26) shown, fragment ions *m/z* 377, 295, 252, and 238 related to the loss of CH_3, C_6H_9, C_3H_7, and CH_3, respectively, were observed in MS/MS analysis of butyl-meta-cycloheptylprodiginine. Poor production of the parent ion at m/z 392.2694 did not allow larger-scale isolation necessary for NMR analysis. However, MS-MS analyses confirmed the presence of butyl-meta-cycloheptylprodiginine as the major component of the mixture.

The fragmentation pattern of the minor component revealed a loss of CH_3 (*m/z* 379), C_9H_{19} (*m/z* 252), and $C_{10}H_{21}$ (*m/z* 238) (Fig. 3E). The fragmentation pattern, the molecular weight and UV-Vis absorbance of this compound with the molecular formula of $C_{25}H_{35}N_3O$, suggested it as the well-known undecylprodiginine. NMR spectroscopy is

almost exclusively used to characterize molecular structures that require a labor-intensive purification process and relatively large amounts of a pure sample. Unusual methyl radical loss combined with weaker methanol loss from each prodiginine is valuable for performing constant neutral loss scans to identify better, faster, and more efficient all prodiginines in a complex mixture without any purification (26).

CONCLUSION

The present study showed the production of chrysomycins B (compound 1), A (compound 2), C (compound 3), and 4'-*O*-acetylated A (compound 4) by *S. chryseus* strain F9 for the first time, which was isolated from soil in Iran. HR-ESI-MS, fragmentation pattern, and database search identified chrysomycins B and C. The structure of chrysomycins A and 4'-*O*-acetylated A was confirmed by 1D and 2D-NMR data. In addition, this study characterized 2 pigments produced by soil-isolated *S. rectiviolaceus* strain F4 using HR-ESI-MS and fragmentation pattern (ESI-MS/MS), which revealed that the major and minor peaks correspond to butyl-meta-cycloheptylprodiginine and undecylprodiginine, respectively. The soil-derived strains showed antimicrobial effects against drug-resistant MRSA and VRE strains.

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Conflicts of interest statement

The authors declared no conflict of interest in this study.

Authors' contributions

S. Ghashghaei, Z. Etemadifar, M.R. Mofid, and H.B. Bode were responsible for the design of the study; S. Ghashghaei and P. Grün performed experiments; S. Ghashghaei wrote the first draft of the manuscript; Y.N. Shi performed NMR analysis under the supervision of H.B. Bode; Y.N. Shi revised the manuscript. All authors read and approved the final version of the manuscript.

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