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STUDY ON ANTIMICROBIAL SECONDARY METABOLITES ISOLATED FROM THREE DERIVED ACTINOMYCETE STRAINS BELONGING TO THE GENUS *Actinoalloteichus* AND *Streptomyces* FROM THE CENTRAL COASTAL REGION OF VIETNAM

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INTRODUCTION

1. Basic of the thesis

The ocean covers 70% of Earth's surface and is home to the planet's greatest biodiversity, harboring 34 out of 36 animal and plant phyla, with over 300,000 known species. The marine environment serves as a rich source of natural compounds, representing an immense reservoir of pharmacological resources awaiting exploration and discovery. The extreme conditions of deep-sea habitats foster the formation of organic compounds with unique chemical structures and valuable biological activities.

Vietnam has a coastline of more than 3,260 km, stretching from north to south with many nearby islands and the two archipelagos Hoang Sa - Truong Sa in the East Sea. These favorable geographical conditions provide Vietnam with immense potential in abundant marine resources, creating a diverse marine ecosystem rich in both species and stock. Vietnam has a strategic focus on developing its marine economy, exploiting natural resources, and researching natural products from the sea. However, research on secondary compounds from Vietnam's marine microorganisms is still in its early stages, with very few published studies, despite the vast diversity of marine microorganisms in the country.

The exploration and search for secondary compounds from marine microorganisms in general, and marine actinomycetes in particular, are currently of significant interest worldwide. Studies on chemical composition and biological activity have shown that secondary compounds from marine actinomycetes possess diverse chemical structures and intriguing biological activities. Numerous secondary compounds derived from marine actinomycetes have been tested for potential applications in medicine, industry, and agriculture.

As part of the key research project "Research on the discovery of anti-tuberculosis and antimicrobial secondary metabolites from marine sediment microorganisms in the South Central Region (Khanh Hoa-Binh Thuan)", the ethyl acetate extracts of actinomycete strains were screened for antimicrobial activity. Among them, three actinomycete strains G631, G666, and G246 showed good antimicrobial activity against 3 - 5 test microorganisms, with MIC values ranging from 2 - 256 µg/ml.

Based on those reasons, project ***"Study on antimicrobial secondary metabolites isolated from three derived actinomycete strains belonging to the genus Actinoalloteichus and Streptomyces from the central coastal region of Vietnam"*** was implemented, **with the aim of** "Searching for

secondary metabolites with antimicrobial activity from three marine actinomycetes (G631, G666, G246) isolated from the Vietnamese sea".

2. The objectives of the thesis :

- Large-scale fermentation of three marine actinobacteria strains with good antimicrobial activity (G631, G666, G246), for biomass production.
- Isolation and chemical structures determination of compounds from the three marine actinomycete strains cultivated in large biomass.
- Determination of antimicrobial activities of isolated compounds.

The thesis includes 140 pages with 29 tables, 69 figures and 96 references.

CHAPTER 1. OVERVIEW

Chapter 1 consists of 28 pages, presenting an overview of research in Vietnam and internationally on the chemical composition and biological activities of actinomycetes and marine actinomycetes.

1.1. Introduction

1.2. *Streptomyces* genus

1.2.1. Streptomyces genus

1.2.2. Secondary metabolites from marine Streptomyces Actinobacteria

1.3. *Actinoalloteichus* genus

1.3.1. Alkaloid compounds isolated from Actinoalloteichus Actinobacteria

1.3.2. The other compounds

1.4. Vietnam publications

CHAPTER 2. MATERIALS AND METHODS

Chapter 2 includes 7 pages, detailing methods of isolation, structure determination, and biological activity assessment methods for antimicrobial screening method.

2.1. Samples

2.1.1. *Actinoalloteichus cyanogriseus* G631

2.1.2. *Streptomyces* sp. G666

2.1.3. *Streptomyces* sp. G246

2.2. Materials and equipment

2.2.1. Chemicals, Equipment, and Methods for Cultivating Biomass on a Large Scale Marine actinobacteria activation and fermentation methods

- The actinomycete strains were cultivated for large-scale biomass production at a 50 kg scale according to Nguyen Van Cach's method (2004) [72].

2.2.2. Method for Extracting Sediment from Biomass Cultivation Products of Actinomycete Strains

2.2.3. Method for Isolating Secondary Compounds

2.2.4. Structural elucidation methods

2.2.5. Antimicrobial screening method

- Antimicrobial activity testing was performed based on the multi-concentration dilution method by Andrews (2001) [75].

CHAPTER 3. EXPERIMENTAL

Chapter 3 includes 19 pages detailing the experimental process: cultivating large amounts of selected actinomycete strains, and isolating primary compounds. and measure physical constants and spectral data of isolated compounds.

3.1. Cultivating biomass of three Actinomycete strains

3.1.1. Solid-state biomass cultivation Actinoalloteichus cyanogriseus G631 strain (50 kg)

3.1.2. Solid-state biomass cultivation Streptomyces sp. G666 strain (50 kg)

3.1.3. Solid-state biomass cultivation Streptomyces sp. G246 strain (50 kg)

3.2. Secondary metabolite isolation from *Actinoalloteichus cyanogriseus* G631

3.2.1. Processing, extract preparation, isolation of the Actinoalloteichus cyanogriseus G631 strain

3.2.2. Physical parameter and spectral data of Actinoalloteichus cyanogriseus G631 isolated compounds

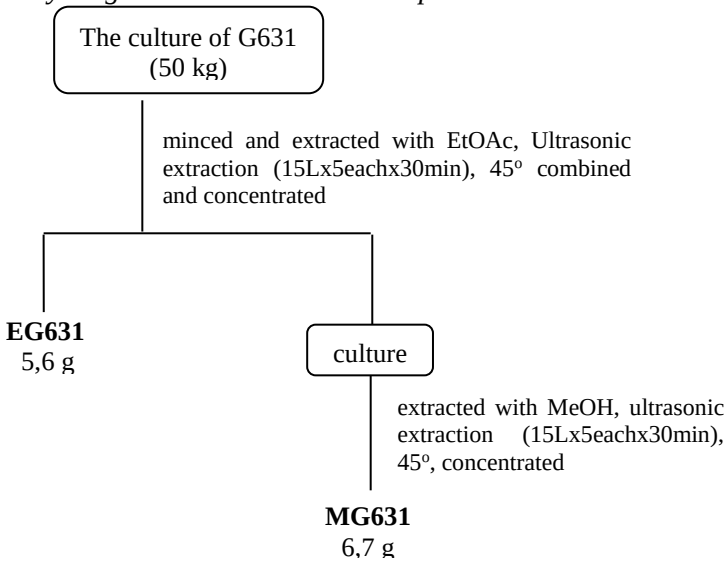


Figure 3.9: the crude extracts from *Actinoalloteichus cyanogriseus* G631

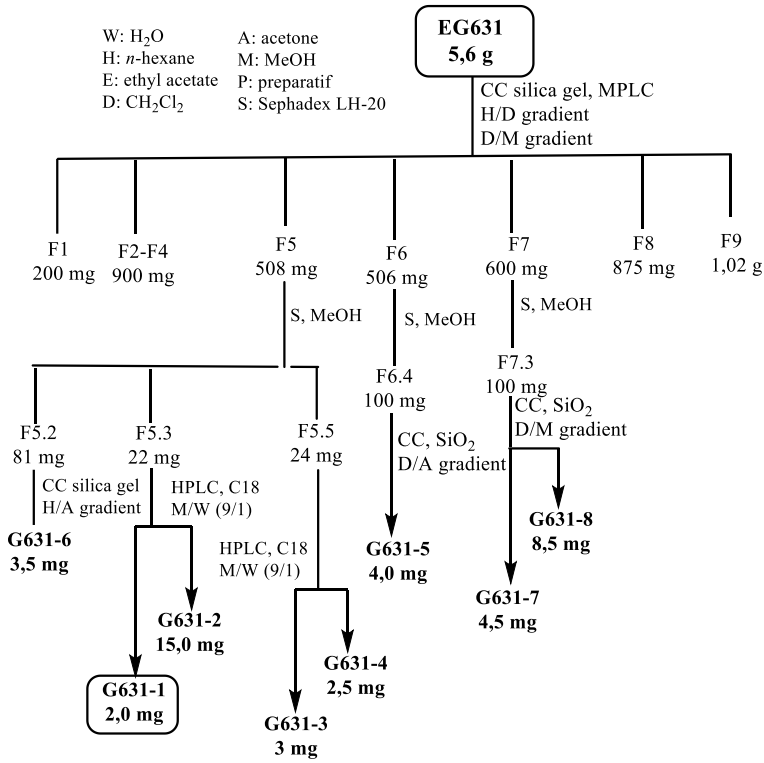


Figure 3.10: Compounds isolated from **G631**

3.3. Secondary metabolite isolation from *Streptomyces* sp. G666

3.3.1. Culture broth extraction, secondary metabolite isolation from extracts of Streptomyces sp. G666

The EtOAc (**EG666**) and MeOH (**MG666**) extracts of 50 kg fermentation broth of *Streptomyces* sp. G666 was achieved with the mass of 22 g and 24 g, respectively.

3.3.2. Physical parameter and spectral data of Streptomyces sp. G666 isolated compounds

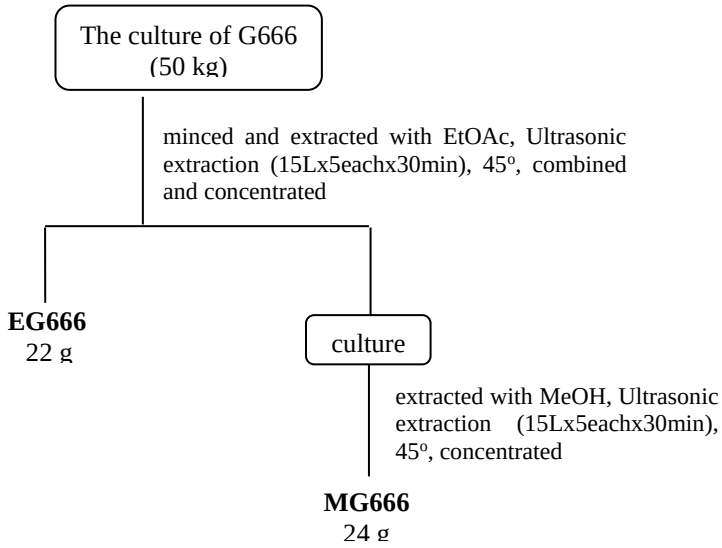


Figure 3.11: the crude extracts from *Streptomyces* sp. G666

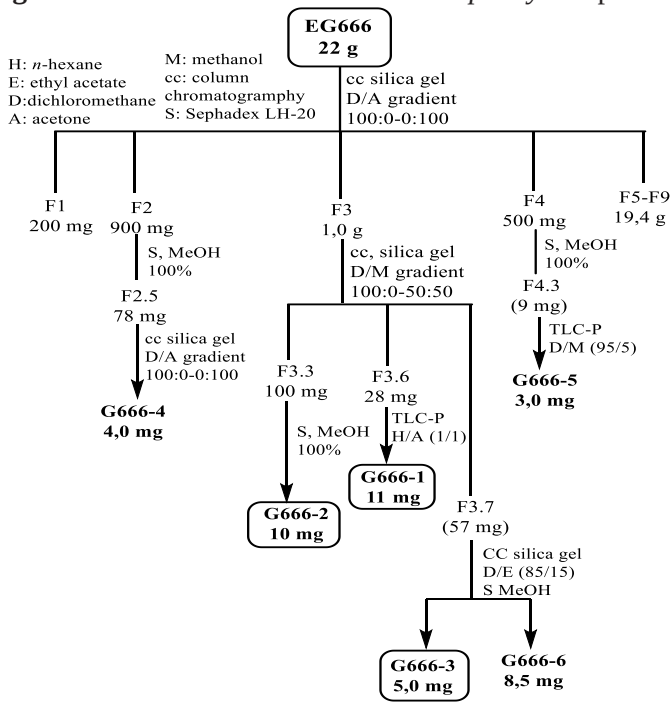


Figure 3.12. Compounds isolated from **EG666**

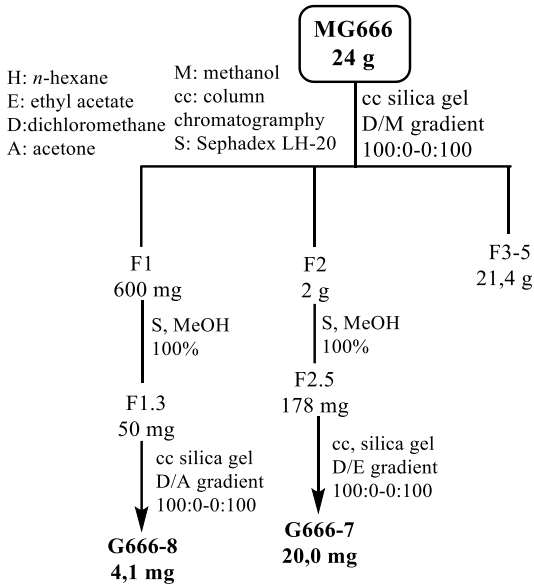


Figure 3.13. Compounds isolated from **MG666**

3.4. Secondary metabolite isolation from *Streptomyces* sp. G246

3.4.1. Culture broth extraction, Secondary metabolite isolation from extracts of Streptomyces sp. G246

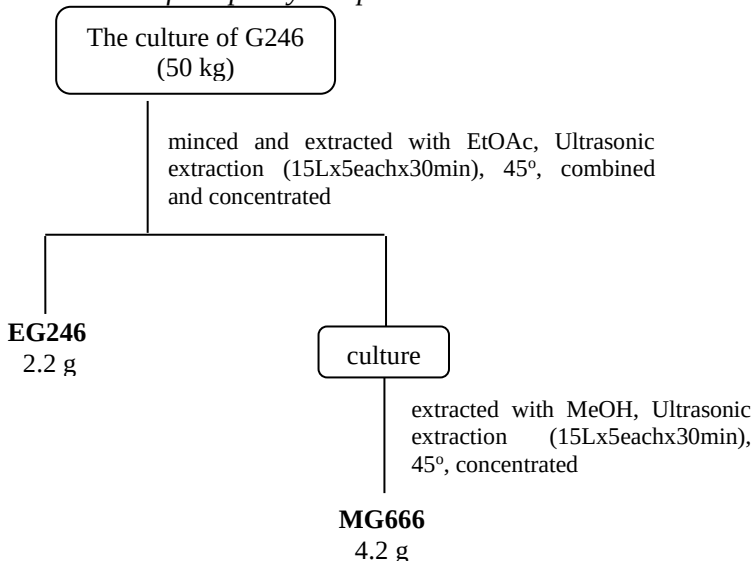


Figure 3.14: the crude extracts from *Streptomyces* sp. G246

The EtOAc (**EG246**) and MeOH (**MG246**) extracts of 50 L fermentation broth of Streptomyces sp. G246 was achieved with the mass of 2,2 g and 4,2 g, respectively.

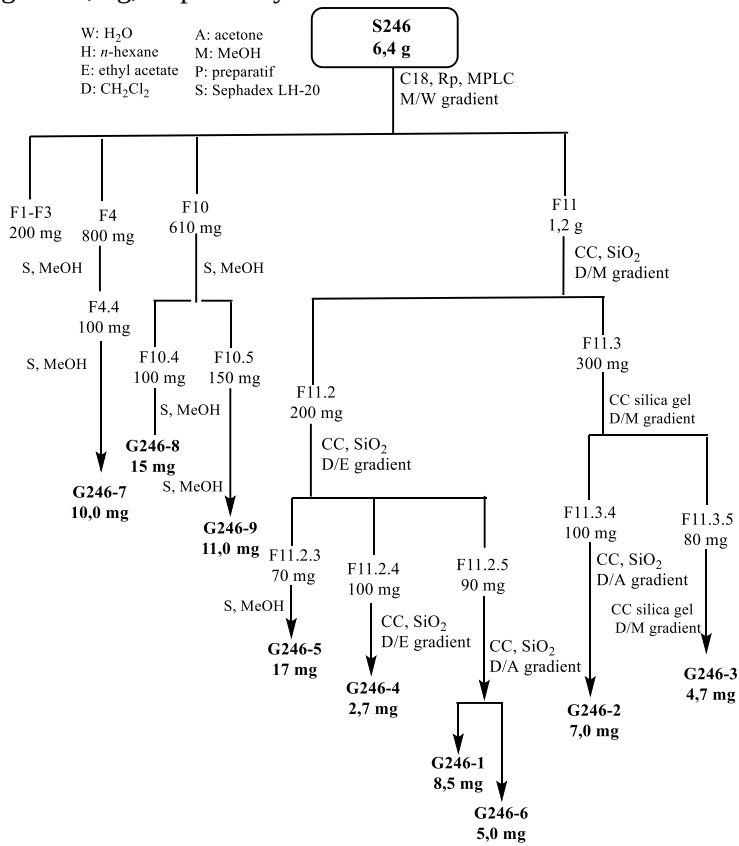


Figure 3.15: Compounds isolated from **G246**

3.4.2. Culture broth extraction, Secondary metabolite isolation from extracts of Streptomyces sp. G246

3.5. Antimicrobial activities of isolated compounds (Table 4.27, 4.28, 4.29)

CHAPTER 4. DISCUSSION OF RESULTS

4.1. Structure elucidation of secondary metabolites isolated from *Actinoalloteichus cyanogriseus G631*

*2.1.1. isocyanogramide (**G631-1**) (new compound)*

Compound **G631-1** was obtained as yellow oil, optically active $[\alpha]^{26}_{D}$ = - 59.3 (*c* 0.6, CH_2Cl_2). HR-ESI MS of **G631-1** showed a pseudomolecular ion peak $[M+Na]^+$ at *m/z* 416.1436, corresponding with the molecular formula $C_{24}H_{21}N_3O_4$ (calcd. for $[C_{24}H_{21}N_3NaO_4]^+$ 438,1424).

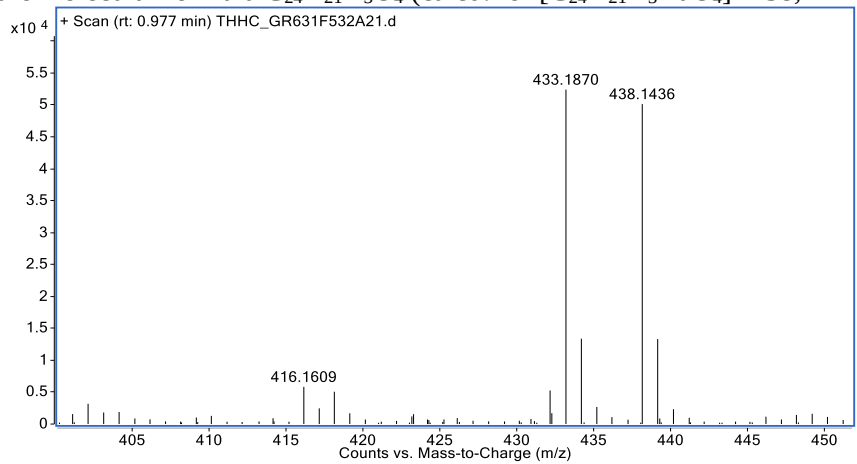


Figure 4.16: HR-ESI-MS Spectrum of **G631-1**

The ^{1}H-NMR spectrum of **1** showed the presence of a mono-substituted phenyl ring at δ_H 7.28 (1H, d, *J* = 7.2, H-6'), 7.32 (2H, d, *J* = 7.8, H-4'/H-8'), 7.37 (2H, d, *J* = 7.2, H-5'/H-7'), one *ortho*-disubstituted benzene ring at δ_H 6.93 (1H, d, *J* = 7.8, H-6), 7.11 (1H, dd, *J* = 7.8, 7.2 Hz, H-4), 7.13 (1H, d, *J* = 7.8 Hz, H-3), 7.40 (1H, dd, *J* = 7.2, 7.8, H-5), two olefinic protons at δH 5.86 (1H, d, *J* = 9.6 Hz, H-1′) and 6.65 (1H, d, *J* = 9.6 Hz, H-2′), suggested the presence of a double bond, three methyl groups at δH 1.95, 3.27, and 3.31 (each 3H, s). The ^{13}C-NMR and HSQC spectra of **G631-1** exhibited signals of 24 carbons, including three carbonyls at δ_C 154.3, 167.5, 170.0, six-non-protonated carbons at δ_C 70.2, 98.6, 124.4, 135.0, 140.2, and 144.9, twelve olefinic methines at δ_C 106.4, 109.2, 115.9, 123.5, 124.3, 128.3, 128.4 × 2, 128.5 × 2, 130.3, and 130.8, and three methyl carbons at δ_C 23.8, 27.1, and 50.2 (**Table 4.4**). Analysis of ^{1}H- and ^{13}C-NMR data of **G631-1** indicated its structure was similar to that of cyanogramide (**G631-2**), a compound that has been isolated from the same species, *Actinoalloteichus cyanogriseus* [60].

The HMBC correlations between H-16 (δ_H 5.84) and C-1 (δ_C 70.2)/C-2 (δ_C 124.4)/C-10 (δ_C 167.5)/C-14 (δ_C 154.3)/C-15 (δ_C 140.2) and between N-methyl protons (δ_H 3.27) and C-7 (δ_C 144.9)/C-9 (δ_C 170.0) proved the presence of spiro rings at C-1 and carbonyl groups at C-9 and

C-10 (**Figure 4.20**). The *ortho*-disubstituted bezene ring at C-2/C-7 was confirmed by HMBC correlations between H-3 (δ_H 7.14) and C-1 (δ_C 70.2)/C-2 (δ_C 124.4)/C-7 (δ_C 144.9), between H-6 (δ_H 6.93) and C-2 (δ_C 124.4)/C-7 (δ_C 144.9) as well as the COSY correlations of H-3 (δ_H 7.14)/H-4 (δ_h 7.11)/H-5 (δ_h 7.40)/H-6 (δ_H 6.93). The prence of phenyl ethenyl was confirmed by HMBC correlations between H-2′ (δ_H 6.65) and C-1′ (δ_C 115.9)/C-3′ (δ_C 135.0)/C-4′ (8′) (δ_C 128.4) and COSY correlations of H-4′ (δ_H 7.32)/H-5′ (δ_H 7.37)/H-6′ (δ_H 7.28)/H-7′ (δ_H 7.37)/H-8′ (δ_H 7.32) and H-1′ (δ_H 5.86)/H-2′ (δ_H 6.65). In addition, the coupling constant between H-1′ and H-2′, J = 9.6 Hz and also NOESY correlations between H-1′ (δ_H 5.86) and H-2′ (δ_H 6.65) suggested the geometry of the double bond of C-1′ and C-2′ is Z [11]. The position of this group at N-13 of tetrahydroimidazole was confirmed by HMBC correlations from H-1′ (δ_H 5.86) and C-12 (δ_C 98.6)/C-14 (δ_C 154.3). Both methyl and methoxy groups at C-12 was proved by HMBC correlations from H-17 (δ_H 1.95) and methoxy proton (δ_H 3.31) to C-12 (δ_C 98.6). Compound **G631-1** has two stereogenic centers, C-1 and C-12. Thus, the experimental ECD has been recorded and shown the negative Cotton effects (CEs) 221, 259 and 309 nm and positive CEs at 238 and 278 nm (**Figure 4.22**), similar to that of cyanogramide (**G631-2**) [60], suggesting the absolute configurations of two stereogenic centers at C-1 and C-12 to be R and S, respectively. Consequently, new structure of compound **G631-1** was elucidated as spirocyclic pirrolo[1,2-c]imidazole, named isocyanogramide

Table 4.4. NMR spectral data of compound **G631-1**

Position	δ_C	Group	δ_H mult. (J in Hz)
1	70.2	C	-
2	124.4	C	-
3	124.3	CH	7.14 d (7.8)
4	123.5	CH	7.11 dd (7.8, 7.2)
5	130.3	CH	7.40 dd (7.8, 7.2)
6	109.2	CH	6.93 d (7.8)
7	144.9	C	-
9	170.0	C	-
10	167.5	C	-
12	98.6	C	-
14	154.3	C	-
15	140.2	C	-
16	106.4	CH	5.84 s
17	23.8	CH_3	1.95 s
18	27.1	CH_3	3.27 s
19	50.2	CH_3	3.31 s

1′	115.9	CH	5.86 d (9.6)
2′	130.8	CH	6.65 d (9.6)
3′	135.0	C	-
4′, 8′	128.4	2CH	7.32 d (7.2)
5′, 7′	128.5	2CH	7.37 t (7.2)
6′	128.3	CH	7.28 t (7.2)

measured in $CDCl_3$; 600 mHz for ^{1}H-NMR and 150 mHz for ^{13}C-NMR

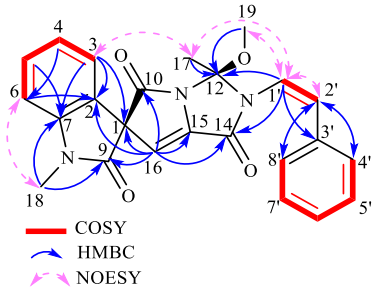


Figure 4.20: Key HMBC, COSY, NOESY correlations of **G631-1**

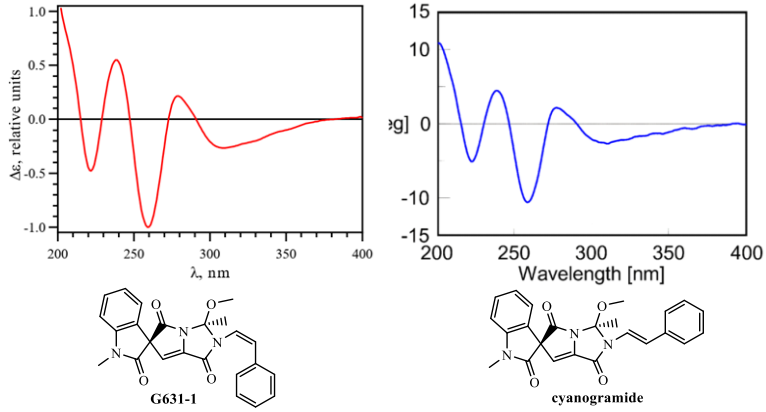
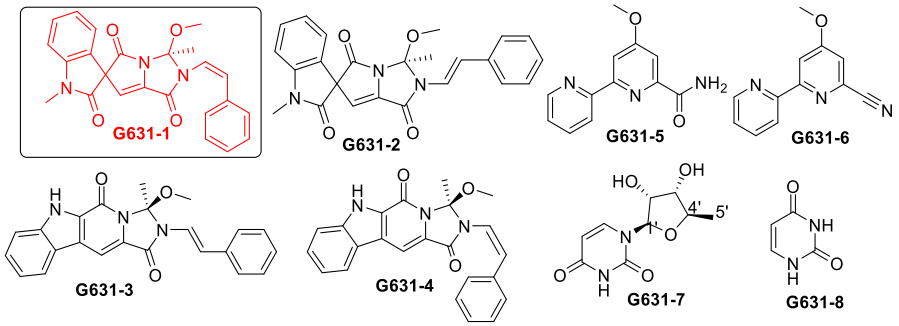


Figure 4.22: CD spectral data of compound **G631-12** and reference compound [60]

*4.1.2. cyanogramide (**G631-2**)*
*4.1.3. marinacarboline F (**G631-3**)*
*4.1.4. marinacarboline H (**G631-4**)*
*4.1.5. caerulomycinonitril (**G631-5**)*
*4.1.6. caerulomycinamide (**G631-6**)*
*4.1.7. 5'-deoxyuridine (**G631-7**)*
*4.1.8. uracil (**G631-8**)*

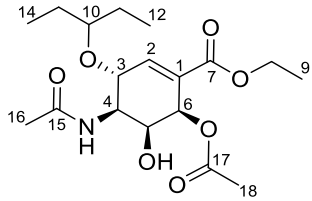


Isolated compounds from strain **G631**

From the culture broth of *Actinoalloteichus cyanogriseus* G631, the isolation and structure elucidation of 8 compounds was achieved, including isocyanogramide (**G631-1**) (new compound), cyanogramide (**G631-2**), marinacarboline F (**G631-3**), marinacarboline H (**G631-4**), caerulomycinamide (**G631-5**), caerulomycinonitril (**G631-6**), 5′-deoxyuridine (**G631-7**), uracil (**G631-8**).

4.2. Structure elucidation of secondary metabolites isolated from *Streptomyces* sp. G666

4.2.1. Streptomine A (G666-1) (new compound)



Compound **G666-1** was isolated as an amorphous solid, and was optically active $[\alpha]^{26}_{D}$ = - 38.2 (*c* 0.6, $CHCl_3$). Its positive HR-ESI mass spectrum showed the proton adduct ion $[M+H]^+$ at *m/z* 372.2026 (calcd for $C_{18}H_{30}NO_7$, 372.2022) which together with ^{13}C-NMR data is consistent with the molecular formula of $C_{18}H_{29}NO_7$. Five degrees of unsaturation was thus deduced for **G666-1**. Analysis of its IR spectrum suggested that it contained one or more hydroxyl groups (3453 cm^{-1}) and carbonyl groups (1645 cm-1).

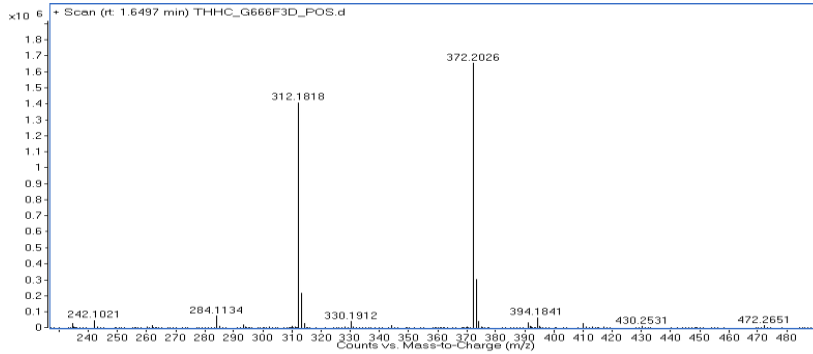


Figure 4.39: HR-ESI-MS Spectrum of **G666-1**

The ^{1}H-NMR spectrum of **G666-1** indicated the presence of five sp^3 methines groups at δ_H 4.32 (1H, dd, J = 2.0, 9.5 Hz, H-3), 3.90 (1H, ddd, J = 2.0, 7.5, 9.5 Hz, H-4), 4.07 (1H, dd, J = 2.0, 2.5 Hz, H-5), 5.65 (1H, d, J = 3.0 Hz, H-6), 3.35 (1H, quint, J = 6.0 Hz. H-10), one olefinic proton at δ_H 7.11 (1H, d, J = 2.0 Hz, H-2), one proton at δ_H 6.18 (1H, d, J = 7.5 Hz, NH), and two acetyl groups at δ_H 2.04 (6H, s, CH_3-16, CH_3-18). Additionally, the resonances of three triplet methyls at δ_H 0.89 (3H, t, J = 7.5 Hz, CH_3-12), 0.94 (3H, t, J = 7.5 Hz, CH_3-14) and 1.27 (3H, t, J = 7.0 Hz, CH_3-9), three methylene groups at δ_H 1.52 (2H, m, CH_2-11), 1.55 (2H, m, CH_2-13), 4.18 (1H, m, H_a-8), 4.25 (1H, m, H_b-8) were also noted. The ^{13}CNMR (**Table 4.12**) and HSQC spectral data of compound **G666-1** indicated the presence of eighteen carbons assigned to three carbonyl groups (δ_C 165.1, 170.1 and 171.5), five methyls, three methylenes, six methines (including one olefinic (δ_C 143.5) and five sp^3 methine groups), and one sp^2 quaternary carbon. The ^{13}C-NMR signals at δ_C 126.7 and 143.5 indicated the presence of one double bond in the structure of **G666-1**. The COSY spectrum of **G666-1** indicated the presence of three spin-spin coupling systems shown in bold lines in Figure 2, including: CH_3-12/CH_2-11/H-10/CH_2-13/CH_3-14, H-2/H-3/H-4/H-5/H-6, and CH_2-8/CH_3-9.

In the HMBC spectrum of **G631-1**, the HMBC cross-peaks of the olefinic proton H-2 (δ_H 7.11) and H-6 (δ_H 5.65) with C-1 (δ_C 126.7) and the carbonyl C-7 (δ_C 165.1) assigned the presence of a cyclohexene ring and the linkage of C-1 with C-7. The chemical shifts of carbons at δ_C 82.2 (C-3), 61.0 (C-8), 70.6 (C-5) and 68.6 (C-6) suggested their linkage to the oxygen atoms and the carbon at δ_C 53.0 (C-4) indicating its linkgae to a nitrogen atom. Additionally, the HMBC correlation of the proton H-10 (δ_H 3.35) with C-3 indicated the connection of C-1 with C-10 of the pentanyl

fragment via an oxygen atom. The presence of the ethyl ester group was defined by the HMBC correlations of the protons CH_2-8 (δ_H 4.18 and 4.25) with C-7 (δ_C 165.1). Finally, the acetyloxy at C-6 was established by the HMBC cross-peak of H-6 (δ_H 5.65) with C-17 (δ_C 170.1), and the position of the acetamide at C-4 was revealed by the HMBC correlations of H-4 (δ_H 3.90) and NH (δ_H 6.18) with C-15 (δ_C 171.5). Thus, the planar structure of compound **G631-1** was established as shown in **Figure 4.45**.

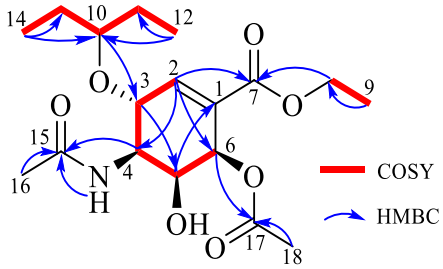


Figure 4.45: Key HMBC, COSY correlations of **G666-1**

The relative configuration of **G631-1** was achieved by the analyses of the proton coupling constants and the NOESY experiment. The proton H-5 had two *gauche* coupling constants (J = 2.0 and 2.2 Hz) assigning the same side orientation for protons H-4, H-5 and H-6. Similarly, an anti-coupling constant (J = 9.5 Hz) between the protons H-3 and H-4 (**Table 4.12**) indicated their axial disposition on the cyclohexene ring. The relative configuration of **1** was confirmed by the NOESY spectrum analysis (**Figure 4.47**). This newly described cyclohexene derivative was named streptomine A.

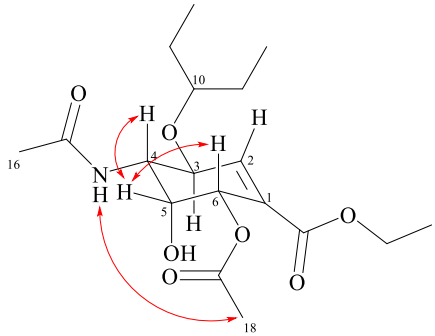


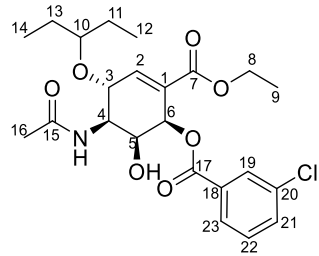
Figure 4.47: Key NOESY correlations of **G666-1**

Table 4.12. NMR spectral data of compound **G666-1**

C	δ_C[a,b]	DEPT	δ_H[a,c] mult. (*J* in Hz)
1	126.7	C	-
2	143.5	CH	7.11 d (2.0)
3	71.6	CH	4.32 dd (2.0, 9.5)
4	53.0	CH	3.90 ddd (2.0, 7.5, 9.5)
5	70.6	CH	4.07 dd (2.0, 2.5)
6	68.6	CH	5.65 d (3.0)
7	165.1	C	-
8	61.0	CH_2	4.18 - 4.25 m
9	14.1	CH_3	1.27 t (7.0)
10	82.2	CH	3.35 quint (6.0)
11	25.6	CH_2	1.52 m
12	9.3	CH_3	0.89 t (7.5)
13	26.3	CH_2	1.55 m
14	9.7	CH_3	0.94 t (7.5)
15	171.5	C	-
16	23.5	CH_3	2.04 s
17	170.1	C	-
18	20.9	CH_3	2.04 s
NH			6.18 d (7.5)

$CDCl_3$; 500 mHz for ^{1}H-NMR and 125 MHz for ^{13}C-NMR

*4.2.2. Streptomine B (**G666-2**) (new compound)*



Compound **G666-2** was isolated as an amorphous solid, with negative optical rotation $[\alpha]^{26}_D$ = -50.1 (*c* 0.25, $CHCl_3$). Its positive HR-ESI-MS showed the proton adduct ion $[M+H]^+$ at *m*/*z* 468.1801; 470,1776 (calcd for $[C_{23}H_{31}{}^{35}ClNO_7]^+$ *m*/*z* 468,1789; $[C_{23}H_{31}{}^{37}ClNO_7]^+$ *m*/*z* 470,1760). Considering the ^{13}C-NMR data, a molecular formula of $C_{23}H_{30}ClNO_7$ was suggested for **G666-2**. Nine degrees of unsaturation was

thus deduced for **G666-2** (**Table 4.13)**. Analysis of its IR spectrum suggested that it contained one or more hydroxyl groups (3453 cm^{-1}) and carbonyl groups (1645 cm-1).

The 1D-NMR spectra of **G666-2** were close to those of **G666-1**. The significant differences between **G666-1** and **G666-2** were the signals of a 3-substituted benzoyl group instead of the acetyl group in the structure of **G666-1**. The linkages of the acetamide at C-4 and the pentanyloxy at C-3 were confirmed by the HMBC correlations of H-4 (δ_H 3.91) and NH (δ_H 6.21) with C-15 (δ_C 171.8), and that of H-10 (δ_H 3.39) with C-3 (δ_C 71.5), respectively. As in the structure of **G666-1**, the ethyl ester group was revealed by the cross-peaks of CH_2-8 (δ_H 4.15 and 4.18) and H-2 (δ_H 7.19) with C-7 (δ_C 165.0) in the HMBC spectrum of **G666-2**. The 3-substituted benzoyloxy group at C-6 was deduced by the HMBC correlations of H-6 (δ_H 5.91), H-19 (δ_H 7.94) and H-23 (δ_H 7.88) with C-17 (δ_C 164.4). The presence of a chlorine in the structure of **G666-2** was suggested by the molecular $[M+H]^+$ and $[M+H+2]^+$ ions with a ratio of 3:1 in its HR-ESI mass spectrum. Since, the carbon C-5 was linked to an oxygen atom as suggested by its chemical shift (δ_C 70.6). The chlorine was thus determined being at C-20 of the benzene ring. The relative configuration of **G666-2** was similar to that of **G666-1**, as established by the proton coupling constants analyses (**Table 4.13**) and the NOESY experiment. Complete analyses of the 2D-NMR spectra confirmed the structure of compound **G666-2**, named streptomine B

Table 4.13. NMR spectral data of compound **G666-2**

C	δ_C[a,b]	DEPT	δ_H[a,c] mult. (*J* in Hz)
1	126,6	C	-
2	144,1	CH	7,19 d (1,0)
3	71,5	CH	4,39 br d (9,5)
4	53,6	CH	3,91 m
5	70,6	CH	4,22 br d (2,5)
6	69,5	CH	5,91 br d (3,5)
7	165,0	C	-
8	61,1	CH_2	4,15-4,18 m
9	14,1	CH_3	1,17 t (7,0)
10	82,5	CH	3,39 m
11	25,5	CH_2	1,54 m
12	9,3	CH_3	0,91 t (7,5)
13	26,3	CH_2	1,57 m

14	9,7	CH_3	0,97 t (7,5)
15	171,8	C	-
16	23,5	CH_3	2,03 s
17	164,4	C	-
18	131,4	C	-
19	129,8	CH	7,94 d (1,0)
20	134,6	C	-
21	133,3	CH	7,52 dd (2,0; 8,0)
22	129,8	CH	7,36 td (2,0; 8,0)
23	128,0	CH	7,88 dd (2,0; 8,0)
HN			6,21 br d (7,5)

a) $CDCl_3$ b)125 MHz c)500 MHz

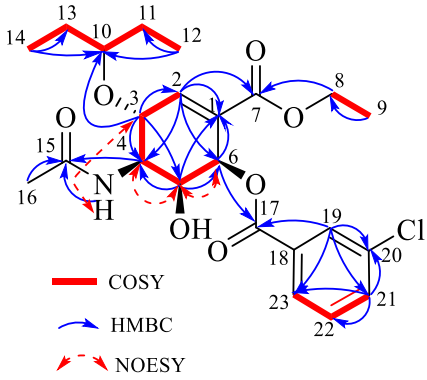
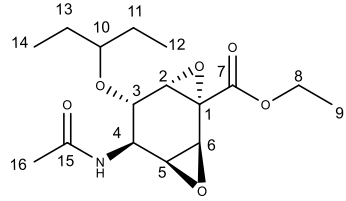


Figure 4.55: Key HMBC, COSY, NOESY correlations of **G666-2**

*4.2.3. Streptomine C (**G666-3**) (new compound)*



Compound **G666-3** was isolated as an amorphous solid, with positive optical rotation $[\alpha]^{26}_D$ = +65.9 (*c* 0.5, $CHCl_3$). Its positive HR-ESI-MS showed the proton adduct ion $[M+H]^+$ at *m/z* 328.1763 (calcd for $[C_{16}H_{25}NO_6]^+$, 328.1760). Together with the ^{13}C-NMR data, a molecular formula of $C_{16}H_{24}NO_6$ was suggested for **G666-3**. Five degrees of unsaturation was thus deduced for **G666-3**. Analyses of 1D NMR data of **G666-3** revealed some similar structural fragments as in **G666-1** and

G666-2, including a pentanyloxy, an acetamide and an ethyl ester groups. Additionally, a connection CH-2 (δ_H 3.65)/CH-3 (δ_H 3.67)/CH-4 (δ_H 4.47)/CH-5 (δ_H 3.37)/CH-6 (δ_H 3.87) was determined by COSY spectrum analysis. HMBC cross-peaks of C-1 (δ_C 57.9) with protons H-2 and H-6 assigned the presence of a cyclohexane ring. The pentanyloxy at C-3 and acetamide at C-4 were indicated by HMBC correlation of C-10 (δ_C 82.1) with H-3 and that of C-15 (δ_C 170.0) with H-4, respectively. The ethyl ester group was confirmed by cross-peaks of C-7 (δ_C 168.0) with CH_2-8 (δ_H 4.32) in the HMBC spectrum. Taking into account of the molecular formula $C_{16}H_{24}NO_6$, two epoxy groups at C-1/C-2 and C-5/C-6 were thus distributed for **G666-3**. Since, the proton H-2 was a broad singlet and H-3 was abroad doublet (J = 9.0 Hz), a *trans*-pseudodiaxial relationship between H-3 and H-4 was determined. Proton H-2 was thus pseudoequatorial on the cyclohexan ring. Furthermore, NOE correlations of H-4 with H-5 and H-6 indicated their orientations on the same side of cyclohexane ring. Complete analyses of 2D-NMR spectra confirmed the structure of compound **G666-3** (**Figure 4.62**), and named streptomine C.

Table 4.14. NMR spectral data of compound **G666-3**

C	**δ_C[a,b]**	**DEPT**	**δ_H[a,c] mult. (J in Hz)**
1	57,9	C	-
2	60,1	CH	3,65 s
3	72,8	CH	3,67 d (9,0)
4	48,1	CH	4,47 dt (1,0; 9,0)
5	55,2	CH	3,37 dd (1,0; 4,5)
6	52,3	CH	3,87 d (4,5)
7	168,0	C	-
8	62,6	CH_2	4,32 m
9	14,1	CH_3	1,34 t (7,0)
10	82,1	CH	3,31 quint (5,5)
11	25,7	CH_2	1.51 m
12	9,1	CH_3	0,88 t (7,5)
13	26,4	CH_2	1,53 m
14	9,6	CH_3	0,93 t (7,5)
15	170,0	C	-
16	23,4	CH_3	2,03 s
H_N			5,6 d (8,5)

[a] $CDCl_3$ [b] 125 MHz [c] 500 MHz

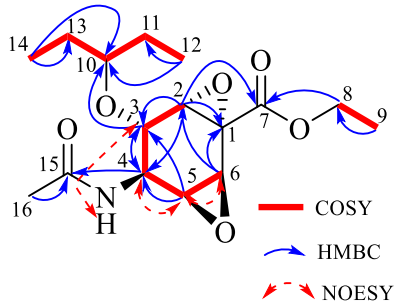


Figure 4.62: Key HMBC and COSY correlations of **G666-3**

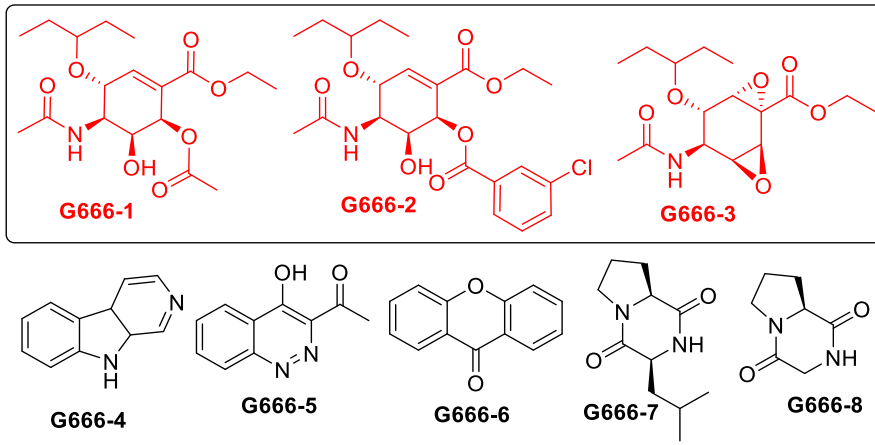
*4.2.4. norharman (**G666-4**)*

*4.2.5. 3-acetyl-4-hydroxycinnoline (**G666-5**)*

*4.2.6. xanthone (**G666-6**)*

*4.2.7. cyclo-(Pro-Leu) (**G666-7**)*

*4.2.8. cyclo-(Pro-Gly) (**G666-8**)*

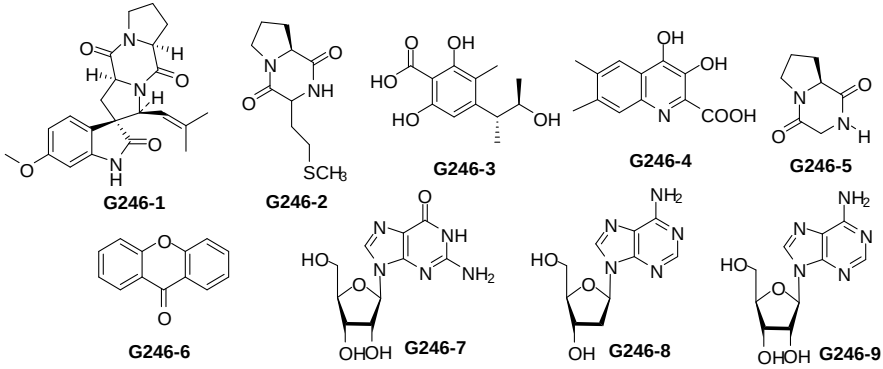


Isolated compounds from strain **G666**

From the culture broth of *Streptomyces* sp. G666, the isolation and structure elucidation of 8 compounds was achieved (Figure), including streptomine A (**G666-1**) (new compound), streptomine B **(G666-2)** (new compound), streptomine C **(G666-3**) (new compound), 9H-pyrido[3,4-b]indole **(G666-4),** 3-acetyl-4-hydroxycinnoline **(G666-5),** xanthone **(G666-6),** cyclo-(leu-Pro) **(G666-7),** cyclo-(Pro-Gly) **(G666-8)**

4.3. Structure elucidation of secondary metabolites isolated from *Streptomyces* sp. G246

*4.3.1. spirotryprostatin A (**G246-1**)*
*4.3.2. cyclo-(Pro-Met) (**G246-2**)*
*4.3.3. phenol A acid (**G246-3**)*
*4.3.4. 3,4-dihydroxy-6,7-dimethyl-quinoline-2-carboxylic (**G246-4**)*
*4.3.5. cyclo-(Pro-Gly) (**G246-5**)*
*4.3.6. xanthone (**G246-6**)*
*4.3.7. guanosine (**G246-7**)*
*4.3.8. adenosine (**G246-8**)*
*4.3.9. 2′-deoxyadenosine (**G246-9**)*



Isolated compounds from strain **G246**

From the culture broth of *Streptomyces* sp. G246, the isolation and structure elucidation of 9 compounds was achieved, including spirotryprostatin A (**G246-1**), cyclo-(Pro-Met) **(G246-2),** phenol A acid (**G246-3**), 3,4-dihydroxy-6,7-dimethyl-quinoline-2-carboxylic (**G246-4**), cyclo-(Pro-Gly) (**G246-5**), xanthone (**G246-6**), guanosine (**G246-7**), 2′-deoxyadenosine (**G246-8**), adenosine (**G246-9**).

4.4. Antimicrobial activities of isolated compounds

All isolated compounds were screened for antimicrobial activities. Obtained results indicated that 17/23 isolated compounds compounds exhibited antimicrobial activities (**Table 4.27**, **Table 4.28** and **Table 4.28**). The pure compounds from the *Streptomyces* strains were tested for activity against 7 pathogenic microorganisms, including 3 Gram-negative bacterial strains: *Escherichia coli* ATCC25922, *Pseudomonas aeruginosa* ATCC27853, *Salmonella enterica* ATCC13076; 3 Gram-positive bacterial strains: *Enterococcus faecalis* ATCC29212, *Staphylococcus aureus*

ATCC25923, *Bacillus cereus* ATCC14579; and 1 fungal strain, *Candida albicans* ATCC10231.

Table 4.27: MIC (µg/ml) values of isolated compounds from *Actinoalloteichus cyanogriseus* G631 strain

N	Comp.	Minimum Inhibitory Concentration - MIC (µg/ml)						
		Gram positive			Gram negative			fungus
		E. faecalis	*S. aureus*	*B. cereus*	*E. coli*	*P. aeruginosa*	*S. enterica*	*C.albicans*
1	**G631-1**	128	64	-	-	-	-	128
2	**G631-2**	128	128	-	32	64	-	32
3	**G631-3**	64	128	-	256	-	-	64
4	**G631-4**	64	128	16	-	64	-	128
5	**G631-5**	128	64	128	-	-	-	32
6	**G631-6**	128	128	-	-	-	-	128
7	**G631-7**	64	256	128	-	-	-	64
8	**G631-8**	-	-	-	-	-	-	-
9	***S**	*256*	*256*	*128*	*32*	*256*	*128*	
10	***C**							32

(S: Streptomycine; C: Cyclohexamide; (-): not active)

Eight secondary metabolites isolated from the strain *Actinoalloteichus cyanogriseus* G631 were evaluated for their antimicrobial activity against seven reference microbial strains. The results indicated that the novel compound **G631-1** exhibited activity against two Gram-positive strains, *E. faecalis* and *S. aureus*, and one fungal strain, *C. albicans*, with minimum inhibitory concentration (MIC) values of 128, 64, and 128 µg/ml, respectively. Compounds **G631-5** and **G631-7** both demonstrated activity against four test microorganisms: *E. faecalis*, *S. aureus*, *B. cereus*, and *C. albicans*, with MIC values ranging from 32 to 256 µg/ml. Compound **G631-4** exhibited activity against five test microorganisms—*E. faecalis*, *S. aureus*, *B. cereus*, *P. aeruginosa*, and *C. albicans*—with MIC values between 16 and 128 µg/ml, showing the strongest activity against *B. cereus* (MIC = 16 µg/ml). Compound **G631-6** selectively exhibited activity against *E. faecalis* and *S. aureus* with an MIC of 128 µg/ml. Compound **G631-2** was active against five reference microorganisms, with notable activity against *E. coli* and *C. albicans* (MIC

= 32 µg/ml). Compound **G631-8** showed no activity against any of the seven reference microorganisms tested.

Table 4.28: Antimicrobial activity of secondary metabolites isolated from *Streptomyces* sp. G666 against reference microorganisms

N	Comp.	MIC (µg/ml)						
		Gram positive			Gram negative			fungus
		E. faecalis	*S. aureus*	*B. cereus*	*E. coli*	*P. aeruginosa*	*S. enterica*	*C.albicans*
1	**G666-1**	64	64	64	-	-	-	16
2	**G666-2**	64	-	-	-	-	-	16
3	**G666-3**	32	64	-	-	-	-	16
4	**G666-4**	-	-	-	128	-	-	-
5	**G666-5**	64	256	256	-	-	-	128
6	**G666-6**	128	256	256	64	256	128	-
7	**G666-7**	-	-	-	-	-	-	-
8	**G666-8**	-	-	-	-	-	-	-
9	***S**	*256*	*256*	*128*	*32*	*256*	*128*	
10	***C**							32

(S: Streptomycine; C: Cyclohexamide; (-): not active)

Three new compounds isolated from *Streptomyces* sp. G666 exhibited good antimicrobial activity against the reference microorganisms. Compound **G666-2** displayed selective activity against two reference strains, *E. faecalis* and *C. albicans*, with minimum inhibitory concentration (MIC) values of 64 and 16 µg/ml, respectively. The novel compound **G666-2** was also active against three Gram-positive strains, *E. faecalis*, *S. aureus*, *B. cereus*, and one fungal strain, *C. albicans*, with MIC values of 64, 64, 64, and 16 µg/ml, respectively. Compound **G666-3** showed activity against two Gram-positive strains, *E. faecalis* and *S. aureus*, and one fungal strain, *C. albicans*, with MIC values of 32, 64, and 16 µg/ml, respectively. All three novel compounds from strain G666 (**G666-1**, **G666-2**, **G666-3**) demonstrated antifungal activity against *Candida albicans* that exceeded that of the positive control compound, cycloheximide. Compound **G666-4** showed selective activity against *E. coli* with an MIC of 64 µg/ml, while compound **G666-6** exhibited activity against six of the seven tested reference microorganisms.

Table 4.29: Antimicrobial activity of secondary metabolites isolated from *Streptomyces* sp. G246 against reference microorganisms

TT	Hợp chất	MIC (µg/ml)						
		Gram positive			Gram negative			fungus
		E. faecalis	*S. aureus*	*B. cereus*	*E. coli*	*P. aeruginosa*	*S. enterica*	*C.albicans*
1	**G246-1**	64	-	128	-	-	-	128
2	**G246-2**	32	-	-	-	-	-	-
3	**G246-3**	-	-	-	-	-	-	-
4	**G246-4**	128	-	-	32	256	64	-
5	**G246-7**	-	-	-	-	-	-	-
6	**G246-8**	32	-	-	-	-	-	64
7	**G246-9**	-	-	-	-	-	-	-
8	***S**	*256*	*256*	*128*	*32*	*256*	*128*	
9	***C**							32

(S: Streptomycine; C: Cyclohexamide; (-): not active)

Among the seven secondary metabolites isolated from *Streptomyces* sp. G246, four compounds demonstrated antimicrobial activity against the reference microorganisms. Compound **G246-1** exhibited activity against two Gram-positive strains, *E. faecalis* and *B. cereus*, and one fungal strain, *C. albicans*, with minimum inhibitory concentration (MIC) values of 64, 128, and 128 µg/ml, respectively. Compound G246-2 displayed selective activity against *E. faecalis* with an MIC of 32 µg/ml. Compound **G246-4** showed activity against all three Gram-negative strains, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella enterica*, with MIC values of 32, 256, and 64 µg/ml, respectively. Compound **G246-8** exhibited strong activity against *E. faecalis* and the fungal strain *C. albicans* with MIC values of 32 and 64 µg/ml, respectively.

Among the 23 compounds tested for antimicrobial activity, 17 compounds showed inhibitory effects on 1 to 7 reference strains, with minimum inhibitory concentration (MIC) values equal to or lower than those of the control antibiotics. Specifically, 16 compounds were active against Gram-positive bacterial strains, 6 compounds against Gram-negative strains, and 13 compounds against the reference fungal strain.

Compound **G666-6** demonstrated broad-spectrum antimicrobial activity, affecting all 6 bacterial strains but showing no activity against the fungal strain. Compounds **G631-2**, **G631-4**, and **G246-4** were active against 4 of the 6 bacterial strains with MIC values equal to or below those of the positive control; however, **G246-4** showed no activity against the fungal strain, while **G631-2** and **G631-4** exhibited MIC values for fungal inhibition comparable to the positive control cycloheximide. Five compounds: **G666-1**, **G631-3**, **G631-5**, and **G631-7** showed activity against 3 of the 6 bacterial strains and were also effective against the fungal strain, with G666-1 displaying the most potent antifungal activity (MIC = 16 μg/ml), significantly lower than cycloheximide. Four compounds, **G666-3**, **G631-1**, **G631-6**, and **G246-1**, exhibited activity against 2 of the 6 bacterial strains with MIC values lower than those of the positive control, streptomycin. Each of these compounds also showed activity against the fungal strain, with **G666-3** showing the strongest activity (MIC = 16 μg/ml). Compounds **G666-2**, **G246-2**, and **G246-8** displayed selective activity against *Enterococcus faecalis*, with MIC values of 64, 32, and 32 μg/ml, respectively, which were significantly lower than that of the positive control, streptomycin. Each of these compounds also demonstrated antifungal activity, with **G666-2** showing the highest activity (MIC = 16 μg/ml). Several compounds exhibited notable antifungal activity against the reference strain *Candida albicans* ATCC10231, with **G666-1**, **G666-2**, and **G666-3** being especially prominent.

CONCLUSIONS

1**.** Strain G666 and G246, active against 5/7 and 3/7 tested microorganisms, were identified in the *Streptomyces* genus; strain G631, active against 5/7 tested microorganisms, was identified as belonging to *Actinoalloteichus* genus. These strains were fermented in solid medium $A1^+$ (without shaking).

2. There were 25 secondary metabolites (including 23 different compounds) isolated from trains *Actinoalloteichus cyanogriseus* G631, *Streptomyces* sp. G666 and *Streptomyces* sp. G246. In which, there are 4 new compounds are streptomine A (**G666-1**), streptomine B (**G666-2**), streptomine C (**G666-3**) and isocyanogramide (**G631-1**). The chemical structures of the new compounds were confirmed by combining modern spectroscopic techniques with comparisons to reference literature.

3. Testing isolated compounds against a panel of microbial pathogens revealed that 18/23 isolates shown inhibition activities against tested pathogens.

RECOMMENDATIONS

1. Further biological characterizations should be conducted for isolated compounds that exhibited antimicrobial activities, such as cytotoxic, anti-inflammatory, and antioxidant properties, with the aim of exploring their potential applications in the future. This is especially relevant for new compounds with unique chemical structures, such as isocyanogramide G631-1, streptomine A G666-1, streptomine B G666-2, and streptomine C G666-3**.**

2. The three actinomycete strains studied in this thesis should be subjected to large-scale biomass production in different media to isolate secondary metabolites with potential biological activity and unique chemical structures. Additionally, with the advancement of science and technology, accessing Vietnam's marine actinomycete resources has become increasingly feasible.

NEW FINDING OF THESIS

1. A new secondary metabolite, named isocyanogramide **G631-1**, has been isolated from the actinomycete strain *Actinoalloteichus cyanogriseus* G631. This compound exhibited weak antimicrobial activity against two Gram-positive strains, *Enterococcus faecalis* and *Staphylococcus aureus*, as well as one fungal strain, *Candida albicans*, with MIC values of 128, 64, and 128 µg/ml, respectively.

2. Three new compounds have been isolated from the actinomycete strain *Streptomyces sp.* G666, were named streptomine A (**G666-1**), streptomine B (**G666-2**), and streptomine C (**G666-3**). These new compounds (**G666-1**, **G666-2**, **G666-3**) exhibited good antimicrobial activity, inhibiting 2 to 4 test microbial strains with MIC values ranging from 16 to 128 µg/ml. Furthermore, these three new compounds demonstrated higher antifungal activity against *Candida albicans* compared to the positive control, cycloheximide, with an MIC value of 16 µg/ml.

3. The selection has been made of three actinomycete strains, G631, G666, and G246 with good biological activity. These strains have been cultivated on large biomass (50 kg scale) to facilitate further studies.

LIST OF THE PUBLICATIONS RELATED TO THE DISSERTATION

1. Do Thi Quynh, Trinh Thi Thanh Van, Nguyen Thuy Linh, Le Thi Hong Minh, Vu Thi Quyen, Brian T. Murphy, Doan Thi Mai Huong and Pham Van Cuong. (2023). A New Alkaloid from Marine-Derived Actinomycete *Actinoalloteichus cyanogriseus* G631, Records of Natural Products, Online: doi.org/10.25135/rnp.412.2305.2776.
2. Do Thi Quynh, Doan Thi Mai Huong, Tran Van Hieu, Truong Bich Ngan, Le Thi Hong Minh, Vu Thi Quyen, Nguyen Thi Hoang Anh, Brian T. Murphy, Phan Van Cuong. (2021). Secondary metabolites produced by marine actinomycete *Streptomyces* sp. G246, Vietnam Journal of Chemistry, 59 (1) (2021)1-8, Online: doi.org/10.15625/2525-2518/58/6/15176.
3. Pham Van Cuong, Doan Thi Mai Huong, Do Thi Quynh, Le Thi Hong Minh, Trinh Thi Thanh Van, Do Thi Quyen, Vu Thi Thu Huyen. The compound streptomine G666A and the method of extracting this compound from the marine actinomycete strain *Streptomyces* sp. G666. Decision accepting valid application No. 11958w/QD-SHTT dated July 15, 2022, application number 1-2022-01427.
4. **Thi Quynh Do**, Thanh Van Trinh Thi, Thi Dao Phi, Thuy Linh Nguyen, Thi Quyen Vu, Hong Minh Le Thi, Brian T. Murphy, Thi Mai Huong Doan, Van Cuong Pham. *New shikimic acid derivatives from the marine-derived actinomycete Streptomyces sp. G666 and their antimicrobial activities*, Chemistry & Biodiversity, 2024 (submitted).