Bioactive Compounds from Marine Streptomyces Sp. by Gas Chromatography-Mass Spectrometry

Tran Vu Phuong*, Phan Van Ha Lam, Cao Ngoc Diep

Biotechnology R&D Institute, Can Tho University, Viet nam

Abstract The objectives of this study were analysis of the secondary metabolite products from extract of marine Streptomyces sp. strain ND7a which isolated from sponges at Ha Tien Sea, Kien Giang province, Vietnam. Six bioactive compounds were identified in the organic solvent hexan-aceton and aceton-methanol. The identification of bioactive chemical compounds is based on the peak area, retention time, molecular weight and molecular formula. GC-MS analysis of Streptomyces sp. strain ND7a revealed the existence of the 2-Pentanone, 4-hydroxy-4-methyl, Cyclohexasiloxane, dodecamethyl, Cycloheptasiloxane, tetradecamethyl, Oxime-, methoxy-phenyl, Hexanedioic acid, bis(2-ethylhexyl) ester and Diisooctyl phthalate.

Keywords bioactive compounds, GC-MS, Ha Tien Sea, Marine sponge, Streptomyces

1. Introduction

In the last decade, marine microorganisms, such as bacteria, microalgae and fungi, have become increasingly important as sources for new bioactive natural products [1-5]. Marine microorganisms have been the important study in recent years because of production of novel metabolites which represent various biological properties such as antiviral, antitumor or antimicrobial activities. These secondary metabolites serve as model systems in discovery of new drugs [6-7]. The studies of the secondary substances produced by marine microorganisms have obtained many significant achievements in the world [8]. Among the secondary metabolites from marine microorganisms, there are many compounds having interesting biological activities that should be useful to development for their pharmaceutical uses. Metabolites from microorganisms is a rapidly growing field, due, at least in part, to the suspicion that a number of metabolites obtained from algae and invertebrates may be produced by associated microorganisms [9]. Meanwhile, the search of bioactive secondary metabolites from marine microorganisms is not widely explored in Vietnam [10-11].

In the past few years, Gas chromatography Mass spectrometry (GC-MS) is used as one of the technological platform for finger print analysis of secondary metabolites in both plant and non-plant species [12]. Taking into consideration the medicinal importance of this plant, the ethyl acetate root extract of medical plant [13] and/or leaves as Neem (Azadirachta Indica A. Zuss) [14], flowers Holarrhena antidysentrica Wall [15] were analyzed using GC-MS. This work will help to identify the bioactive components. GC-MS is the best technique to identify bioactive constituents of long chain hydrocarbons, alcohols, acids, ester, alkaloids, steroids, amino and nitro compound etc.

In the course of our screening program, the EtOAc extract of a Streptomyces sp. from marine sponge of Ha Tien Sea, Kien Giang province, Vietnam exhibited an inhibition activity against Salmonella typhymurium, Echerichia coli, Bacillus cereus and Candida albicans. In this paper, we reported the isolation and structural elucidation of secondary metabolites from the cultures broth of Streptomyces sp. in two kinds of organic solvent. The present study
was aimed to identify the chemical constituents in ethyl acetate extract of marine actinomycetes were analyzed by the GC-MS technique.

2. Materials and methods

2.1. Actinomycete material

The marine sponge was collected in Ha Tien Bay – Kien Giang province in April 2016. The sponge sample (1 g) was added to the 10 mL of sterile sea water in a conical flask. The flask was agitated for about one hour. The marine sponge was filtered and the filtrate was serially diluted to obtain $10^{-1}$ to $10^{-7}$ dilutions using the sterilized sea water. An aliquot of 100 µL of each dilution was spread on the media. Different media like Starch Casein Agar (SCA) was used for isolation of actinomycetes. The media containing 50% of sterile sea water were supplemented with rifampicin (5 µg/mL) and nystatin (25 µg/mL) (Himedia Mumbai) to inhibit bacterial and fungal contamination, respectively. The petriplates were incubated up to 3 weeks at 28°C. The isolated discrete colonies were observed and used for identification.

The obtained strain *Streptomyces* sp. was identified by using 16S rRNA gene sequencing method. The universal primers including forward primer, 5'- AGA GTT TGA TCA TGG CTC A-3'; and reverse primer, 5'- AAG GAG GTG ATC CAG CC- 3', were used for amplifying nearly full length of 16S rRNA gene sequence (about 1500 bp.). The obtained sequence was analyzed by comparing with bacterial 16S rRNA sequences in GenBank by Blastn, which showed 99% similarity with *Streptomyces* sp. 2011 (GenBank Accession No. JF751041.1).

2.2. Fermentation, extraction and isolation

*Streptomyces* sp. strain ND7c was cultured in 250 ml flasks at 30°C for 24 hours with shaking at 150 rpm. Fermentation was carried out in 100 L fermenter with 50 L medium 2216 and 10% bacterial inoculum at 30°C for 52 hours. Neutral pH was maintained automatically by NaOH or HCl 1N. The obtained culture broth (50 L) was extracted with ethyl acetate (25 L x 3 times). The combined organic solutions were then decanted, filtered and concentrated under reduced pressure to yield 5.2 g of crude extract which was chromatographed on a silica gel column using a gradient of 1 - 100% acetone in hexane to afford three fractions F1-3, after that it was continuously chromatographed on a silica gel column using a gradient of 1 - 100% acetone in methanol to afford three fractions F4-6. Therefore, six fractions were received from 2 kinds of organic solvent (hexane – acetone and acetone – methanol).

2.3. GC/MS analysis

The samples were analysed at GC/MS of Chemistry Laboratoty, Department of Chemistry, College of Natural Science, Can Tho University. GC-MS analysis of the sample was carried out using Shimadzu Thermo with column TG-SQC; 15m x 0.25mm x 0.25µm. Helium was used as the carrier gas and the temperature programming was set as follows:

<table>
<thead>
<tr>
<th>Speed (°C/min)</th>
<th>Temperature (°C)</th>
<th>Keep (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>50</td>
<td>1.00</td>
</tr>
<tr>
<td>Ramp 1</td>
<td>2.00</td>
<td>70</td>
</tr>
<tr>
<td>Ramp 2</td>
<td>10.00</td>
<td>150</td>
</tr>
<tr>
<td>Ramp 3</td>
<td>10.00</td>
<td>250</td>
</tr>
<tr>
<td>Total time</td>
<td>43 minutes</td>
<td></td>
</tr>
</tbody>
</table>

10 µl sample was injected with split less mode. Mass spectra was recorded over 35-400 amu range with electron impact ionization energy 70 eV, total running time for a sample was 43 min. Quantitative determination were made by relating respective peak areas to TIC areas from GC-MS.

3. Results and Discussion

GC-MS analysis of compounds from extract of Streptomyces sp. strain ND7a with organic solvent Hexan-Aceton as shown in Figure 1.
Figure 1: GC-MS chromatogram of extract of Streptomyces sp. strain ND7a in organic solvent Hexan-Aceton

Chromatogram GC-MS analysis of hexan-aceton extract of Streptomyces sp. strain ND7a showed the presence of three major peaks (Table 1) and the components corresponding to the peaks were determined as follows. The first set up peak was determined to be 2-Pentanone, 4-hydroxy-4-methyl (Figure 2).

Figure 2: Mass spectrum of 2-Pentanone, 4-hydroxy-4-methyl with retention time (RT) = 4.64
The second peak indicated to be Cyclohexasiloxane, dodecamethyl (Figure 3) and the next peaks considered to be Cycloheptasiloxane, tetradecamethyl (Figure 4).

![Mass spectrum of Cyclohexasiloxane, dodecamethyl](image1)

**Figure 3:** Mass spectrum of Cyclohexasiloxane, dodecamethyl with retention time (RT) = 13.115

![Mass spectrum of Cycloheptasiloxane, tetradecamethyl](image2)

**Figure 4:** Mass spectrum of Cycloheptasiloxane, tetradecamethyl with retention time (RT) = 15.803

<table>
<thead>
<tr>
<th>S/N</th>
<th>RT (min)</th>
<th>Name of the compound</th>
<th>Molecular weight (g/mol)</th>
<th>Molecular formula</th>
<th>Peak (%)</th>
<th>Bioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.64</td>
<td>2-Pentanone, 4-hydroxy-4-methyl</td>
<td>116.3</td>
<td>C₆H₁₂O₂</td>
<td>93.00</td>
<td>Antimicrobial</td>
</tr>
<tr>
<td>2</td>
<td>13.115</td>
<td>Cyclohexasiloxane, dodecamethyl</td>
<td>444</td>
<td>C₁₂H₃₆O₆Si₆</td>
<td>97.50</td>
<td>Antifungal properties</td>
</tr>
<tr>
<td>3</td>
<td>15.803</td>
<td>Cycloheptasiloxane, tetradecamethyl</td>
<td>518</td>
<td>C₁₄H₄₂O₇Si₇</td>
<td>97.40</td>
<td>Antiperspirants and deodorants, Antibacterial, Antifungal.</td>
</tr>
</tbody>
</table>
GC-MS analysis of compounds from extract of *Streptomyces* sp. strain ND7a with organic solvent Aceton-Methanol as shown in Figure 5.

Figure 5: GC-MS chromatogram of extract of *Streptomyces* sp. strain ND7a in organic solvent Aceton-Methanol

Chromatogram GC-MS analysis of hexan-aceton extract of *Streptomyces* sp. strain ND7a showed the presence of three major peaks (Table 2) and the components corresponding to the peaks were determined as follows. The first set up peak was determined to be Oxime-, methoxy-phenyl (Figure 6).

Figure 6: Mass spectrum of Oxime-, methoxy-phenyl with retention time (RT) = 5.99

The second peak indicated to be Hexanedioic acid, bis(2-ethylhexyl) ester (Figure 7) and the next peaks considered to be Diisooctyl phthalate (Figure 8).

Figure 7: Mass spectrum of Hexanedioic acid, bis(2-ethylhexyl) ester with retention time (RT) = 30.82
Figure 8: Mass spectrum of Diisooctyl phthalate with retention time (RT) = 33.07

Table 2: Major compounds identified in extract of *Streptomyces* sp. strain ND7a (acetone-methanol)

<table>
<thead>
<tr>
<th>S/N</th>
<th>RT (min)</th>
<th>Name of the compound</th>
<th>Molecular weight (g/mol)</th>
<th>Molecular formula</th>
<th>Peak (%)</th>
<th>Bioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.99</td>
<td>Oxime-, methoxy-phenyl</td>
<td>151</td>
<td>C₉H₁₂NO₂</td>
<td>84.5</td>
<td>Antifungal, Antibacterial, Anticancer and Antitumor</td>
</tr>
<tr>
<td>2</td>
<td>30.82</td>
<td>Hexanedioic acid, bis(2-ethylhexyl) ester</td>
<td>370</td>
<td>C₂₂H₄₂O₄</td>
<td>52.1</td>
<td>Antimicrobial activity, Antioxidant, antiproliferative</td>
</tr>
<tr>
<td>3</td>
<td>33.07</td>
<td>Diisooctyl phthalate</td>
<td>390</td>
<td>C₂₄H₄₀O₄</td>
<td>20.0</td>
<td>Antifungal, Antibacterial, Antiviral and Antioxidant activities</td>
</tr>
</tbody>
</table>

Shettima *et al.* [16] discovered 2-pentanone – 4-hydroxy-4-methyl acid diethyl phthalate in *Guiera senegalensis* J.F. Gmel which is used in West African Ethnomedicine for treating diarrhoea, dysentery, malaria, cough and microbial infections; and 2-pentanone-4-hydroxy-4-methyl acid diethyl phthalate is one in nine compounds from the ethyl acetate root extract of *Guiera senegalensis* were identified by Gas-chromatography–Mass spectrometry (GC-MS) analysis. The biological activities of each of the identified phytocomponents range from antimicrobial, antioxidant and antitumoral activities. Cyclohexasiloxane dodecamethyl and Cycloheptasiloxane tetradeca-methyl were analysed by GC-MS technique from the ethanol extract of stem bark of *Cola nitida* from the University of Ibadan, Nigeria. Cola has been reported to have very high medicinal values; it has been attributed to the treatment of ringworm, scabies, gonorrhea, dysentery and opthalmia [17]. Worthy of note also is the reports of Kim [18] and Jayeola for soft drinks production [19] that alluded to Cola used as a remedy for whooping cough and asthma. *Cola nitida* possess antifungal properties against dermatophytes (*Trichophyton rubrum, Trichophyton tonsurans*) and *Candida albicans* as reported by Sewanu *et al.* [20]. The antimicrobial activities of n-Hexane extract and 5 Column Chromatography fractions, of *Azadirachta indica* A. juss (Neem) leaves, showed antimicrobial activities against human pathogenic bacteria (*Salmonella typhi*) and yeast fungus (*Candida albicans*). Akpuaka *et al.* [14] used GC/MS identified 45 bioactive compounds in the n-Hexane extract of *Azadirachta indica* leaves out of which 33 have antifungal activity and Oxime-, methoxy-phenyl- and Diisooctyl phthalate were identified were two in 33 bioactive compounds. Both of compounds are Antifungal, Antibacterial, Anticancer, Antitumor [19] and Antifungal, Antibacterial, Antiviral and Antioxidant activities [21]. Paramanantham and Murugesan [15] and Kadhim *et al.* [22] identified Hexanedioic acid, bis(2-ethylhexyl) ester, is bioactive compound, antimicrobial activity, it was extracted from flower of *Holarrhena antidiscentrica* Wall and *Vitis vinifera* tree.
4. Conclusion
In the present study six compounds from the ethyl acetate root extract of *Streptomyces* sp. strain ND7a were identified by Gas-chromatography–Mass spectrometry (GC-MS) analysis in two kinds of organic solvent (hexan-aceton and aceton-methanol). The biological activities of each of the identified components range from antimicrobial, antioxidant and antitumoral activities. The nature of the identified compounds are mostly organic acids. The research findings have shown that the is extensively rich in secondary metabolites and they have been reported as bioactive compounds and they have been used in the world.

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References


