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BIOACTIVE COMPOUNDS OF STREPTOMYCES QUS7 ISOLATE ETHYL ACETATE EXTRACT FROM SEDIMENT IN ALDIWANYIA RIVER

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Abstract:
This study was subjected for the first time to *Streptomyces sp*. QUS7 has been identified and described using morphological and biochemical characteristics from the sediment of the Al-Diwaniya River in southern Iraq. Extract of the ethyl acetate was tested with GC-MS. The strain was identified as the genus Streptomyces based on biochemical, microbiological, and morphological characteristics. 2,4-bis(1,1-dimethylethyl)-phenol, pentadecanoic acid, tetradecene, methyl ester, 14-methyl-, 1-H-cycloprop(e)azulen-7-ol, decahydro-1,1,7-trimethyl-, 4-methylene, hexadecene-2-pentadecanone, 6,10,14-trimethyl, hexadec Nonacosan Additional. the study is needed to clarify the antimicrobial agent's structure and purification.

Keywords: Streptomyces, QUS7, Ethyl acetate, sediment, Al-diwanyia, river

INTRODUCTION:

The inappropriate use of antibiotics causes MDR organisms. MDR traits are assigned to resistant bacteria based on European CDCP, at least one agent in three or more antimicrobial classifications [1].

Other types of antibiotics must be investigated to combat MDR infections [2, 3]. Actinomycetes, for example, can be used to produce new compounds for biological applications. Antimicrobial drugs must be developed due to their distinct mode of action. Gram-positive bacteria with hyphal structures, such as actinomycetes, are the most essential microorganisms in the environment for producing secondary metabolites such as antibiotics [4. 5].

The most specific antibiotic producers are Streptomyces and Micromonospora [5]. Streptomyces produces 80% natural products and has unrivaled secondary metabolite production ability [6].

Streptomyces sp. can produce a diverse range of biologically significant secondary metabolites, including antimicrobial activity, that can be used to treat humans and animals. Over 7,000 metabolites are thought to be produced by these bacteria. Members of the genus Streptomyces are responsible for about 80% of these infections. Antibiotics, antiparasitics, anticancer, insecticides, herbicides, and other bioactive metabolites are all produced by *Streptomyces spp* [7].

Aims os the current study which identification and descrition of Streptomyces QUS7 isolate by biochemical testes from the sediment of the Al-Diwaniya River. Extract of the ethyl acetate and some the other substances. the study is needed to clarify the antimicrobial agent's structure and purification

MATERIAL AND METHODS:

1-Isolation:

A sample of air-dried sediment was dried for one hour at 100 ° C [8]. The treated sediment was dissolved in 1 g of sterile, distilled water and mixed thoroughly by vortex for the starch-casein-nitrate agar. The homogenate sample was applied to the medium's surface, after which a serially diluted sample of 0.01 ml was done add (10-4 dilution), then for 24 hours of incubation at 37°C. CaCO3 = 0.002 %, MgSO4 = 0.002 % for three weeks, the plates were placed at 30°C, Then placed at 37°C. The different strains subculturing on extract dextrose agar (yeast extract malt) to make (ISP-2) slants.

2. Crude Extract Preparation:

During the preliminary screening, *Streptomyces sp.* isolate QUS7 was identified and cultured for seven days at 28 C on potato dextrose agar PDA (DifcoTM Company USA). After cutting the agar into 1 cm pieces, mixed with 250 ml of ethyl acetate before being placed in top-capped bottles with 500 ml. Overnight at 30 C, the agar and solvent solutions were agitated at 150 rpm in a revolving shaker. The mixtures were put in the centrifuge at 15,000 rpm for 10 minutes to separate the supernatants (Avanti J-26 XPI, Beckman Company, United States). Whatman No.1 filter papers (AL-bertr) were used to filter the extracts prior to drying with rotary evaporation. As a crude sample, the concentrated extract powder was

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prepared and kept at 20°C for chromatographic analysis; a crude extract was diluted in 50% ethyl acetate.

3-Gas Chromatography-Mass Spectrometry technique:

To determine the purity of the crude extract, a Shimadzu GC-17A was used in conjunction with the Shimadzu GC-MS-QP5050A system. A 30 ml, 0.25 mm I.D., 0.25 m film thickness column was used. Low bleed Phenomenex Zebron ZBFFAP Phenomenex Zebron's bonded Polyethylene Glycol fused capillary column. A split ratio injection was performed with a split ratio of 20:1. A flow rate of 0.7 ml/min of helium was employed as the transport gas. If a pre-programmed start time of 3 minutes was assumed for the experiment, the column temp was 70°C. There was a dissolvable delay of 5.75 minutes between the detector and input temperatures of 230 and 250 degrees Celsius (solvent delay).

RESULTS:

1-Morphological characteristic

Colonies have widely been used to identify Streptomyces isolate [9]. SDJ10 isolate of Streptomyces sp. Put in starch casein agar (SCA) and kept at 37 °C. The result of morphological properties revealed is filamentous organisms, gram-positive, whitish and substrate mycelium, and smooth colony. On the reverse side, the colonies appeared creamy. The morphologies were assessed, with isolate identified as Gram-positive filamentous and branching bacilli.

2-Physiological and biochemical characteristics:

Streptomyces spp characteristics are shown in (Table 1). The isolate is Gram-positive with long filamentous shapes. The optimum temperature for streptomyces growth was 28 °C, indicating that isolate is mesophilic. For pH, however, the optimum pH was pH 7 [10]. On the other hand, the optimal NaCl concentration for the Streptomyces isolate's growth was 1%. NaCl concentration has been reported to influence the growth of Streptomyces to isolate, as similarly observed in this study [11]. The isolate was positive for oxidase, starch agar, urea hydrolysis, skim milk agar, mannitol, xylose, D-galactose, D-Fructose, L-Arabinose, and citrate utilization. The isolate was negative for xanthine agar, Rhamnose, and VP. These findings are in accord with the findings of [10]

Table (1): *Streptomyces* sp. QUS7 Isolate physiological and biochemical characteristics

Characteristics	***Streptomyces sp.***
Gram stain	Positive
Shape and growth	Long filamentous
Optimum temperature	28°C
temperature of growth	25°C - 45°C
Growth pH	7
Range of pH for growth	4 – 10
Growth in the presence of NaCl	1-8%
Growth under anaerobic condition	-
Motility	Non-motile
Oxidase	+
Starch hydrolysis	+
Casein hydrolysis	+
Urea hydrolysis	+
Skim milk agar	+
Gelatin Hydrolysis	+
Xanthine Agar	-
Mannitol	+
Indole	-
Catalase	+
Xylose	+
Rhamnose	-
D-Galactose	+
D-Fructose	+
L-Arabinose	+
VP	-

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Citrate utilization	+
TSI	Alk/Alk

SCANNING ELECTRON MICROSCOPY (SEM) OF Streptomyces sp. QUS7 Isolate:

Scanning-electron microscopic examination was performed on Streptomyces sp. QUS7 isolates to assess the spore surface and chain for morphological features (Figures 1), Streptomyces sp. QUS7 displayed widespread branching of the mycelia and spiraling chains, comprising spin-surfaced spores. These morphological features observed under SEM are similar to those reported in earlier related studies [12].

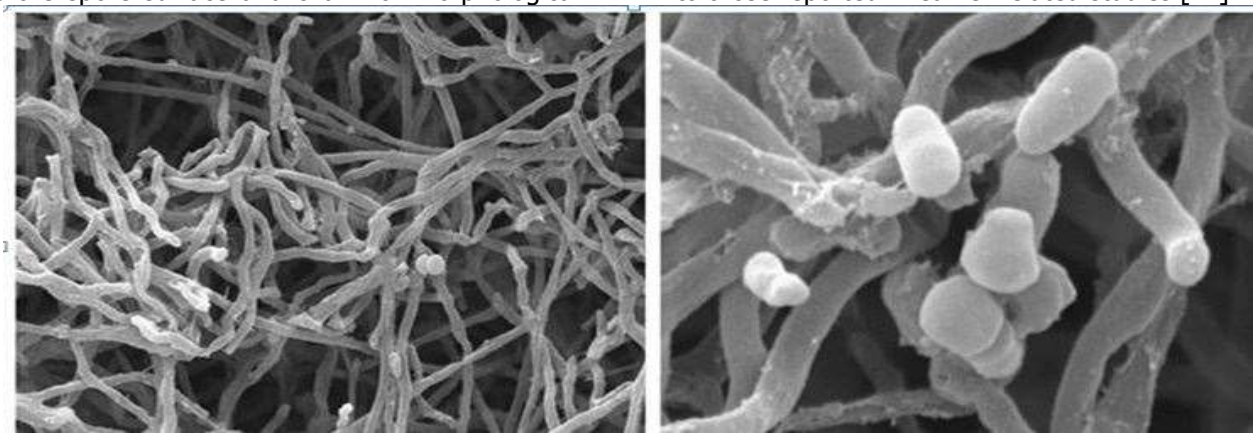

Figure (1): Electron microscopy of Streptomyces sp. QUS7. Mag. 20.00 KX

GC/MS analysis of EA extract:

Forty compounds belonging to various classes were found in Streptomyces sp. QUS7 crude extract by GC-MS analysis and had varying 490 Nayer M. Fahmy, 2020 percentages of peak areas. Benzenepropanoic acid, Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl-4-hydroxy)-methyl ester, Benzenepropanoic acid, Hexadecanone 2-Pentadecanone, 6,10,14 Trimethyl, 3,5-bis(1,1-dimethylethyl-4-hydroxy)-methyl ester, Hexadecanoic acid methyl ester Nonacosane, Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl-4-hydroxy) ester Hexadecanoic acid, methyl ester Eicosene, 9, Nonacosane, (Table 2 ,Figure 2).

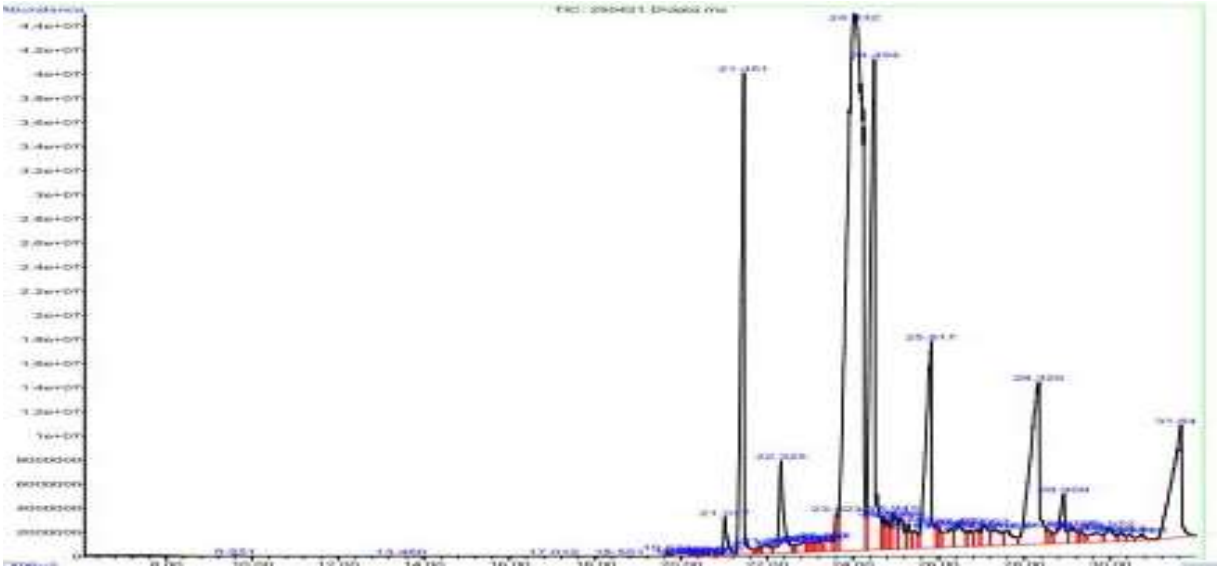

Figure (2): Chromatogram of Ethyl Acetate Streptomyces sp. QUS7 extract

Table (2): GC-MS Identified Components of Streptomyces sp. QUS77 Extract (Compounds are listed in ascending high percentage)

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Compound	Nature of the compound	Biological activity
Phenol, 2,4-bis(1,1-dimethylethyl)	Phenol	antimicrobial (13), antifungal antioxidant (14) antitumor(15; 16)
Tetradecene	Alkenes	Antifungal and Antibacterial (14)
Pentadecanoic acid, 14-methyl-,methyl ester	Fatty acid methyl ester	Antimicrobial, antifungal (17)
1H-Cycloprop(e)azulen-7-ol, decahydro-1,1,7- trimethyl-4-methylene-,	Aromatic Compound	Antifungal, larvicidal agent and Insecticidal (18)
Hexadecene		Antibacterial, Antifungal, and antioxidant activity (19)
2-Pentadecanone, 6,10,14-trimethyl		Antibacterial activity against Gram +ve and Gram-ve bacteria (20)
Nonacosane	Alkenes	Antibacterial activity (21)
Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl-4-hydroxy-, methyl) ester	Aromatic Compound	Antifungal, antioxidant,(22)
Hexadecanoic acid, methyl ester	Fatty acid methyl ester	Anti-oxidant, decrease blood cholesterol, anti-inflammatory (23)
Eicosene	Alkane	Antibacterial, antitumor, antifungal, cytotoxic(24)
9-Octadecenoic acid (Z)-methyl ester	Unsaturated fatty acid methyl ester	Antioxidant, anti cancer (25; 23)
Docosane	Alkane	Antibacterial (18)
Dodecanoic acid methyl este	Fatty acid methyl ester	Antibacterial, antiviral, antifungal(26)

DISCUSSION:

The use of GC-MS for determination of the compounds present in Streptomyces ethyl acetate extract depending on molecular weight, peak areas, and molecular formula (Selvin, et al., 2009). The use of GC-MS for the determination of compounds in Streptomyces sp. QUS77 ethyl acetate extract was depended on the peak areas (Nandhini et al., 2015).

It has been shown and substantiated that the peak areas are directly proportional to the amount of the chemical that is present in the active band (Nandhini et al., 2015). In this work, we employed GC-MS to do the analysis, and we used peak area %, retention duration, and molecular formula to identify 13 different chemicals.

The major constituents were 2,4-bis(1,1-dimethylethyl)-phenol, pentadecanoic acid, tetradecene, methyl ester, 14-methyl-, 1-H-cycloprop(e)azulen-7-ol, decahydro-1,1,7-trimethyl-, 4-methylene, hexadecene-2-pentadecanone, 6,10,14-trimethyl, hexadec Nonacosan. This findings agreement with studies (Nandhini et al., 2015). compounds in Streptomyces were found to be present. In addition, the existence of additional minute peaks in the spectrum is a hint that the extract may include other chemical substances that have not yet been discovered. It is possible that the antibacterial activity is caused by either the main components on their own or the major constituents in conjunction with the minor elements. These studies demonstrated the opportunities that are embedded in marine Streptomyces as a possible source of novel and more efficient antibiotic agents (Narendhran et al., 2014).

Some the extracted compounds have biological effects (Thirumalairaj et al., 2015) that have antibacterial and antifungal effects (Sengupta et al., 2015).

In several reports found that the antioxidant effect to extracted compounds (Sudha and Masilamani, 2013; Asghar 2011), while some the other studies found theses compounds have antibacterial and antifungal activity such as Phenol, 2,4-bis (1,1-dimethylethyl), decahydro-1,1,7- trimethyl-4-methylene-, dodecanoic acid methyl ester, n-tridecanoic acid methyl ester, 1-octadecanol, eicosane, and octadecanoic acid methyl ester (Nandhini et al. 2015; Thirumalairaj et al. 2015).

Many studies found that the antioxidant effect to the phenol, 2,4-bis (1,1-dimethylethyl), phenol 4,6-di(1,1-dimethylethyl)-2-methyl, 1-hexadecene, hexadecanoic acid, 9-octadecenoic acid, hexadecanoic acid, and butyl ester (Sudha & Masilamani 2013).

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(Sudha & Masilamani 2013) found that Phenol, 2,4-bis(1,1-dimethylethyl), 1-octadecene, 9-octadecenoic acid, eicosane and 9-octadecenoic acid have anti-cancer effects. (Rahdary & Sobati 2012) found that Hexadecanoic acid causes reduce the cholesterol level in the serum and can decrease the cyclooxygenase enzymes.

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