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# Effects of *streptomyces rochi* Biosurfactants on Pathogenic *Staphylococcus aureus* and *Pseudomonas aeruginosa.*

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**Abstract:** Fifty soil samples were collected from soils contaminated and uncontaminated with hydrocarbons. Six isolates belonging to *Streptomyces rochei* were diagnosed, 8% from contaminated soil and 6% from uncontaminated soil. Isolates were diagnosed depending on the study of 16s rDNA compared to standard isolates within the National Center for Biotechnology Information site. 28 smears of wounds and 12 smears of burns were collected, including *Staphylococcus aureus* isolates (35.7%) and *Pseudomonas aeruginosa* (16.6%). The isolates of *Staphylococcus aureus* and *Pseudomonas aeruginosa* showed multiple resistance to antibiotics, Oxacillin, Erythromycin, Nalidixic acid, and Tetracycline. Isolate *Streptomyces rochei* 19 showed the ability to produce biosurfactants that have antagonistic properties against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. In addition, the biosurfactant production from *Streptomyces rochei* 19 were non-toxic to the potential growth of *Brassica oleracea* seeds at 1.5 and 50 mg/cm$^3$. The biosurfactant was diagnosed as Lipopeptide using thin layer chromatography and GC-Mass technique.

**Keywords:** *Streptomyces rochei*, Antimicrobial, Biosurfactant, GC-Mass.

## INTRODUCTION

The genus of Streptomyces represents the largest group within Actinobacteria (Prescott et al., 2018). In general, the members of the Streptomycetaceae family can be distinguished by means including morphological, physiological traits, the chemical composition of the cell wall, the type of peptidoglycan, and type of fatty acid chains (Madhaiyan et al., 2016). Molecular methods including GC ratio, 16s rRNA sequence techniques, and hybridization methods were also implied (Law et al., 2019). It is characterized by being a gram-positive filamentous bacterium, non-acid fast stain, heterotrophic, able to grow in different environments, catalase positive, nitrate reduces to nitrite and has the ability to hydrolyze adenine, ascoline, casein, and starch (Chen et al., 2018). The filament is divided into one level to form chains of nonmoving 3-5 spores with a smooth, spinal, or hair-like surface (Maleki et al., 2013). The cell wall contains peptidoglycan with large amounts of L-diaminopimelic acid and does not contain mycolic acid (van der Aart et al., 2018). Streptomyces species have the ability to produce a distinctive earthy odor because it produces volatile compounds called geosmin (Juttner & Watson, 2007). It produces many pigments which are responsible for giving color to the substratum and aerial mycelium. There are roughly 550 species identified belonging to this genus and more than 3000 strains which have confused the classifiers in

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finding their diagnostic tables (Lee & Whang, 2014). The Streptomyces genus is known to be a rich source of antibiotics (Alsammak & Alhyaly, 2019), accounting for 75% of the world's naturally produced antibiotics (Barka et al., 2016). *Streptomyces rochei* was first isolated from soil in Russia. It has the ability to produce many secondary metabolites including borrelidin, the surface, or interfacial tension between two liquid phases such as oil/water or air/liquid interfaces (Gudiña et al., 2016). The chemically synthesized surfactants have a wide range of applications in industries (Kaczorek et al., 2013). However, the chemical synthesized surfactants are expensive and are causing environmental pollution. Biosurfactants are natural products produced by microorganisms. The Biosurfactants have unique properties which differ from chemically synthesized surfactants (Nitschke & Silva, 2018). Biosurfactants are environmentally friendly, biodegradable, and have good anti-microbial activities at extreme temperatures, pH, and salinity (Thavasi et al., 2011). These advantages allow biosurfactants to be a substitute for chemically synthesized surfactants. Biosurfactants can be classified depending on their chemical structures into glycolipids, lipopeptides, polysacchride-protein complexes, phospholipids, fatty acids, and neutral lipids (Geetha et al., 2018). The aim of this study was to study antibacterial activities of S*treptomyces rochei* that has the ability to produce biosurfactants.

## MATERIALS AND METHODS

### Isolation of Streptomyces

Fifty soil samples were collected from different sites in Mosul Nineveh city. These included samples contaminated with hydrocarbons (from the generator's area and the oil refineries of Qayarah). Non-polluted soil samples from domestic and public gardens were also included. The samples were taken at a depth of about (2-5) cm. The dilutions of soil were done by taking 1g soil/9 $cm^3$ sterile distilled water, mixed with a vortex for 100 seconds to obtain cell preparation/$cm^3$, and then performing successive decimals to reach dilution to $10^{-4}$. $1cm^3$ of dilution $10^{-3}$ and $10^{-4}$ were taken and distributed by pouring on Starch Casein Agar. The plates were incubated aerobically for 7-10 days at a temperature of 30ºC (Gebreyohannes et al., 2013).

**Isolation of *Staphylococcus aureus* and *Pseudomonas aeruginosa*:** Forty swabs of burns and skin wounds were taken from random people using transport peptone water. The samples were then cultured on the nutrient agar medium to isolate Pseudomonas and the mannitol salt agar to isolate Staphylococcus. Oxacillin resistance screening agar base (ORSAB) was used to isolate methicillin-resistant Staphylococcus (MRSA). The plates were incubated aerobically at 37ºC for 24 hours (Murray et al., 2003).

**Molecular diagnoses of *Streptomyces rochei* and *Staphylococcus aureus:*** Isolation of bacterial DNA using a DNA extraction kit (Wizard ® genomic DNA purification kit) from Promega Company.

### PCR to investigate the 16s rDNA gene

Primers: lyophilized universal primers were used by Promega as in table (1). Primers were dissolved in sterile distilled water to obtain a concentration of 10 picomol/µl.

**Electrophoresis method:** Genetic DNA was detected using electrophoresis on agarose gel electrophoresis at a concentration of 1% agarose. The power supply was adjusted at 50 volts for 80 minutes, and the agarose gel was stained with Diamond $^{TM}$ Nucleic acid.

**Table: (1)**. The sequence of 16s rDNA primers

| The name of gene used | 5′→3′ | Primer | Product size Bp | Reference |
|---|---|---|---|---|
| 16s rDNA | AGAGTTTGATCCTGGCTCAG | 27 Upstream | 1350 | (Cotarlet et al., 2010) |
| | GACGGGCGGTGTGTAC | 1392 Downstream | | (Lane, 1991) |

**PCR reactions to investigate the 16s rDNA gene:** A total of 25µl for all samples containing

the following ingredients were prepared by mixing 12.5µl of green master mix, 1µl from each forward and reverse primers, 4µl of DNA template extracts at a concentration of 50ng/µl, and 6.5 µl of nuclease-free water in a 0.2 Eppendorf tube. Ingredients were blended well by making a spin for 10 seconds with a cold centrifuge, and then the tubes were inserted into the thermocycler device by using the following program:
Min 5 94ºC 1 Cycle
Sec 30 94ºC
Min 1 55ºC 30 Cycles
Min 1 72ºC
Min 5 72ºC 1 Cycle. (Cotarlet et al., 2010)
The PCR products were detected by electrophoresis in 2% agarose at 50 volts for 80 min. The gel was examined after staining with Diamond $^{TM}$ Nucleic acid for 45 minutes using Ultraviolet light to detect the 16s rDNA genes at the site of 1350 base pairs. The molecular size of the DNA fragment was estimated by comparing the band size with the DNA ladder (Sambrook & Russell, 2001).

**Sequences analysis for 16s rDNA gene:** The PCR amplification products were sent with the forward and reverse primers to the Micro-gene laboratory in Korea, where the genotype sequences of the gene 16s rDNA were determined. The results were analyzed using the Basic Local Alignment search tool (BLAST) program, available at the National Center for Biotechnology Information (NCBI) on the site ( http://www.ncbi.nlm.nih.gor).

**Primary screening of the ability of *Streptomyces rochei* 19 to produce biosurfactants**
**First: hemolytic activity:** Hemolysis was car

ried out on plates of blood agar medium supplemented with (5%) human blood. The blood agar plates were inoculated and incubated at 37ºC for 7 days. Plates were examined for a clear zone around the colonies (Carrillo et al., 1996).

**Second: modified drop collapse metho:** Drops of crude oil were dropped as a thin layer on the surface of a glass slide. A 10µl drop of the supernatant of seven days Streptomyces was cultured in maltose Yeast extract broth by cooling centrifuge at 10000 rpm/min for 15 min on the slide. The droplet shape was then observed one minute later. The negative result is explained by the fact the droplet remains convex and the same size, while the positive result is when the drop collapses and becomes flat with a slightly larger size. The result was compared by adding water as a negative control and SDS as a positive control. The test was performed with three replications per isolation (Plaza et al., 2006).

**Third: lipase production:** For lipase activity, Luria-Bertani agar plates supplemented with (1%) of an olive oil emulsion were made (pH of the medium adjusted 7.0) inoculated with a fresh culture of Streptomyces and incubated at 37ºC for seven days. After incubation, plates were observed for a clear zone of hydrolysis around the colony (Maniyar et al., 2011).

**Forth: oil spreading assay method:** 50µl of distilled water was added to the Petri dish, Followed by adding 20µl of crude oil on the surface of the water. 10µl of supernatant was added onto the center of the oil film. The diameter of the clear zone on the oil surface was measured and compared with control using cultured broth (Cornea et al., 2016).

**Fifth- Emulsification index (E24):** Samples were determined by adding 2ml of paraffin oil and 2ml of the cell-free broth in a test tube. Vortexed at high speed for 2 min and allowed to stand for 24h, the percentage of the Emulsification index was calculated using the following equation (Abouseoud et al., 2008).
E24=Total height of emulsion formed (cm)*100/total height of solution (cm)

**Productivity medium used for biosurfactant from *Streptomyces rochei:*** Two media were used for productivity:
1-Maltose Yeast Extract Broth (MYE) supplemented 1% of olive oil (Kokare et al., 2007). The broth culture was incubated aerobically at

30ºC in a shaker incubator at 120 rpm for seven days. 2-Starch nitrate medium, the broth cultures were incubated aerobically at 30ºC in a shaker incubator at 120 rpm for seven days (Shubhrasekhar et al., 2013).

**Biosurfactant Extraction :** First method: the liquid productive culture was incubated for seven days, centrifuged at 100 rpm/min for 15 minutes, the precipitate was discarded, and the supernatant was taken and mixed with the same volume of solvent chloroform-methanol (2:1 v/v) using separating funnels. The bottom layer containing the active substance was taken and evaporated with rotary evaporation, and a dry material was stored in the refrigerator until use (Kiran et al., 2017).

Second method: the culture samples were centrifuged at 14000 rpm/min for 20 min to remove the bacterial cells. 1M HCl was added to the supernatant to reach pH 2.0. The precipitate was collected by centrifugation at 12000 rpm/min for 30 min at 4ºC to obtain crude biosurfactant. The crude biosurfactant was dissolved in deionized water and adjusted to a 7.0 pH and extracted by a solvent having a chloroform: methanol (2:1 v/v), the organic phase (chloroform layer) was evaporated using a rotary evaporator to remove the solvent chloroform. A dry material was stored in the refrigerator until use (Deepa et al., 2015).

**Antibiotics Sensitivity:** By using the Kirby-Bauer method, the isolates of *Staphylococcus aureu*s and *Pseudomonas aeruginosa* at 18 hours with the concentration of ($1.5*10^8$ cell/$cm^3$) compared to McFarland tubes were inoculated by swab on Muller-Hinton Agar. The sensitivity to the following antibiotics: Tetracycline 10µg, Oxacillin 5µg, Carbencillin 25µg, Erythromycin 10µg, Nalidixic acid 30µg, and Gentamicin 10µg was studied, the plates were incubated at 37ºC for 24 hours and then the diameter of the inhibition zone was measured (Winn et al., 2006).

**Antimicrobial activity of biosurfactant:** A sterile filter paper was taken and immersed in biosurfactant extract solution by sterile forceps and put in Muller Hinton Agar inoculated with *Staphylococcus aureu*s and *Pseudomonas aeruginosa* at the age of 18 hours with the concentration of $1.5*10^8$ cell/$cm^3$(compared to McFarland tubes). The plates were incubated at 37ºC for 24 hours (Gebreyohannes et al., 2013).

**Determination of biosurfactant toxicity:** The phytotoxicity test was used to investigate the action of the biosurfactant on cabbage seeds (*Brassica oleracea* L.). Different concentration of biosurfactant solution (1.5 and 50 mg/ml), and control solution (distilled water), were tested. The biosurfactant samples were placed in Petri dishes, each with ten seeds. The plates were incubated for five days at 27ºC. The number of germinated seeds was then counted and the length of the roots was measured from the point of transition from the hypocotyl to the extremity of the root (Yerushalmi et al., 1998). The germination index (GI), which is one of the most commonly used ways to characterize the phytotoxicity of a compound, was calculated as follows:

%GI = (% of seed germination) x (% of root growth): 100

in which % of seed germination = (% of germination in the extract): (% of germination in the control) x 100; and % of root growth = (mean growth in the extract): (mean growth in the control) x 100. The analyses were performed in triplicate.

**Separation of biosurfactant extract using a thin layer chromatography technique**:The obtained extract was separated using 20 $cm^3$ X 20 $cm^3$ silica chromatography plates, the extract was mixed with the solvent DMSO, and the samples were left at room temperature or dried using a hair dryer. The separation solution consisted of chloroform / methanol/ water (65:25: 4, by vol.) (Symmank et al., 2002). The flow rate was calculated for all separated spots according to the following law:

Flow rate = distance traveled by the extract/distance traveled by the solvent.

The compounds separated by TLC were visualized by spraying with 0.5% ninhydrin (w/v, in water or ethanol) to identify the free amino groups. The plates were heated at 110º C for 5 min until the appearance of a reddish-brown color (Bezza & Chirwa, 2015).

**Characterization of biosurfactant by Gas chromatography-mass spectrometry (GC/MS)**: The chloroform: methanol extract was analyzed by gas chromatography-mass spectrometry (GC/MS). The spectrum of the crude component was compared with the spectrum of the known components in the National Institute Standard and Technology (NIST) library. The name, molecular formula, weight, and chemical structure of the components of the test materials were identified.

**Phylogenetic relationships:** The evolutionary relationship among the six *Streptomyces rochei* was obtained using the Unweighted Pair Group Method with Arithmetic mean (UPGM) within Molecular Evolutionary Genetics Analysis version 7 (MEGA7) using the Maximum Composite Likelihood method based on (Tamura et al., 2004). The strains were submitted to NCBI with the accession number for each strain.

## RESULTS AND ISCUSSIONS

**Isolation:** Twenty-four isolates belonging to Streptomyces species were isolated, as shown in table (2), the percentage of *Streptomyces rochei* from hydrocarbon contaminated soils was 8% and uncontaminated soils was 16%, which are considered to be the main bacteria of the soil (Loganathan Karthik & Rao, 2010). The morphological characteristics of the colonies and cells and their adhesion to the nutritional medium and odor production of isolates suspected to belong to the genus of Streptomyces were studied (G Al-Sammak et al., 2009). Species of the genus Streptomyces are difficult to characterize, as indicated by the researcher Holt and his colleagues in 1994 (Holt et al., 1994). While the isolation rate of Pseudomonas from burns was 16.6%, Staphylococcus from wounds was 35.7%. Six isolates belonging to *Streptomyces rochei* were genetically diagnosed using 16s rDNA.

**Table: (2).** Percentage of bacteria isolated from environmental and clinical samples

| Isolated bacteria | Isolation rate % | Number of isolates | Number of samples | Type of samples |
|---|---|---|---|---|
| *Streptomyces rochei* | 8 | 2 | 25 hydrocarbonate soil | Soil |
| | 16 | 4 | 25 uncontaminated soil | |
| Staphylococcus | 35.7 | 10 | 28 swabs | Wound |
| Pseudomonas | 16.6 | 2 | 12 swabs | Burns |

**Phylogenetic relationship:** By using 16s rDNA for molecular diagnosis of *Streptomyces rochei*, the *Streptomyces rochei* strains have shown similarity between (99.42-99.7) % to reference strains in the NCBI. The isolates where clustered together in the phylogenetic tree at 99.4%, as shown in figure (1).

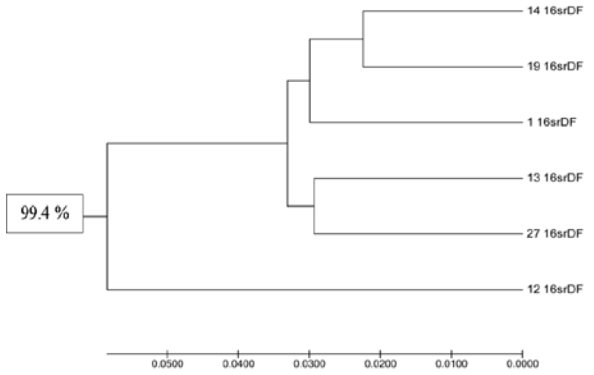


**Figure: (1).** Phylogenetic tree of the 16S rDNA *Streptomyces rochei* based on UPGMA method with aid of MEGA 7. Program.

Depending on some morphological trials and biochemical tests, in addition to the use of 16s rDNA, four isolates were supposed to be *Staphylococcus aureus*, namely 1,6,7,9. These strains showed β-hemolysis, positive for catalase test, and fermentation of mannitol sugar. A special medium (ORSAB) was used to isolate the *Staphylococcus aureus* drug resistance. This medium contains two types of antibiotics,

Oxacillin and Polymixin B, with the presence of sodium chloride salt in the concentration of 5.5%. The methicillin resistance *Staphylococcus aureus,* colonies appeared in blue color on the ORSAB medium and yellow color on the Mannitol salt agar (Simor et al., 2001). While *Pseudomonas aeruginosa* cells showed positive for oxidase and citrate, β-hemolysis, producing pyocyanin stain and foul smell similar to rotting fruit smell as this characteristic was used in the initial diagnosis of the species. And the diagnosis of species was ensured by using an API 20E strip. The *Staphylococcus aureus* and *Pseudomonas aeruginosa* species showed resistance in 100% to Oxacillin, Erythromycin, Carbenicillin, and Nalidixic acid, as shown in table (3). The *Staphylococcus aureus* resistance to β-Lactam antibiotics was due to the ability of the species to produce penicillin-binding protein 2a (PBP2a) encoded by the *mec*-A gene located on the mobile gene element (MGE) that has a low roll of β-Lactam antagonists. In recent years, the species has been resistant to many antibiotics, including Macrolides, Aminoglycoside, Quinolones, and Tetracycline, because resistance genes are carried on plasmids and transposons, which can be transmitted between species and other gram-positive bacteria (Akpaka et al., 2017). While the resistance of the *Pseudomonas aeruginosa* is due to its possession of pumping systems for many antibiotics, in addition to the plasmids responsible for resistance, the mutation also has a role in the resistance (Lister et al., 2009).

**Hemolytic activity**: Initial investigation on the ability of *Streptomyces rochei* isolates to produce biosurfactants using four methods. Six *Streptomyces rochei* isolates were selected, as shown in table (4). The isolates showed complete hemolysis, and the use of this test was based on the fact that biosurfactants can cause the analysis of erythrocytes. The researcher (Chakraborty et al., 2009) pointed out that there is a relationship between the effectiveness of blood hydrolysis and the production of biosurfactants. The use of hydrolysis of blood is a primary indicator to search for the production of biosurfactants.

**Table: (3) .**The resistance of *Staphylococcus aureus* and *Pseudomonas eruginosa* to antibiotics

| No. Isolation | Bacteria | Vancomycin (VA) 30 | Oxacillin (OX) 5 | Erythromycin (E) 10 | Carbencillin (PY) 25 | Gentamicin (CN) 10 | Nalidixic acid (NA) 30 | Tetracyclin e (TE)10 |
|---|---|---|---|---|---|---|---|---|
| 1 | *Staph.aureus* 1 | S | R | R | R | S | R | R |
| 2 | *Staph.aureus* 6 | M | R | R | R | R | R | R |
| 3 | *Staph.aureus* 7 | S | R | R | R | S | R | R |
| 4 | *Staph.aureus* 9 | S | R | R | R | M | R | R |
| 5 | *Staph.aureus* 10 | M | R | R | R | R | R | R |
| 6 | *Staph.aureus* 17 | S | R | R | R | R | R | S |
| 7 | *P.aeruginosa* 1 | R | R | R | R | R | R | R |
| 8 | *P.aeruginosa* 2 | R | R | R | R | R | R | R |

R= resistance S= sensitive M= moderate

**Table: (4).** Initial investigation of *Streptomyces rochei* to produce biosurfactants.

| Accession number in NCBI | Strains | Hydrolysis on blood agar | Lipolysis medium | Modified drop collapse test | Oil dispersion test |
|---|---|---|---|---|---|
| MN589659 | *Streptomyces rochei*1 | + | - | - | + |
| MN594555 | *Streptomyces rochei* 12 | + | + | - | + |
| MN594553 | *Streptomyces rochei* 13 | + | + | - | + |
| MN594540 | *Streptomyces rochei* 14 | + | - | - | + |
| MN589658 | *Streptomyces rochei* 19 | + | + | + | + |
| MN594530 | *Streptomyces rochei* 27 | + | + | - | + |
| | Percentage % | 100 | 66.6 | 16.6 | 100 |

**Lipase production**: Some strains showed a positive result for the lipolysis test. The result showed a clear zone around the colony, evidence of its ownership of the lipase enzyme, which works to breakdown fat to produce fatty acids, as the production of this enzyme is associated with the production of biosurfactant (Deepa et al., 2015). The results were con-

sistent with the study of (Kokare et al., 2007), in which of the 80 strains of the genus Streptomyces, only 56 were positive for the lipase test.

**Modified drop collapse method:** Most of the isolates showed a negative result to the modified drop collapse test, even though it gave a positive result in the hemolysis test. The negative result may be due to the fact that the test is hyposensitivity, in which biosurfactant concentration must be rather high in the filtrate to cause the droplet to collapse (Walter et al., 2010). This method (modified drop collapse) is an easy, fast, and non-specialized for a particular type of biosurfactant and needs a small amount of the sample, easy to perform, and does not need special requirements (Khopade et al., 2012).

**Oil spreading assay method:** All strains that gave good hemolytic activity gave good efficacy to the oil dispersion test. The diameter of the clear zone is in direct correlation with the effectiveness of biosurfactant. When a drop of biosurfactant was added to the oil layer on the water, a clear zone was observed (Figure 2) as in the study of (Al-Safar & E.G.AL-Sammak, 2014)

. The organisms producing biosurfactants alone can disperse oil, as noted by the research (Youssef et al., 2004). The oil dispersion test has been shown to be more sensitive than the method of testing the modified drop collapse in detecting the presence of biosurfactant in the filtrate of the bacterial culture (Ainon et al., 2013). In order to know the productivity of biosurfactants, many researchers use two or more methods to search for biosurfactants. Relying on one method is inappropriate in diagnosing all kinds of biosurfactant, therefore, a set of tests is used to find the product object (Bodour & Maier, 2003).

**Figure (2).** Oil spreading assay: zone of clearance by crude biosurfactant from *Streptomyces rochei*.

**The production of biosurfactants from *Streptomyces rochei* strain 19:** The results showed that the insoluble carbon source in the medium, olive oil, gave the highest increase in productivity over the water-soluble carbon source, glycerol, by observing the diagonal sizes to test the dispersion of oil as indicated by (Abouseoud et al., 2007), probably due to the bacteria's exploitation of to the source of dissolved carbon at high speed in the production of energy and biomass and the lack of the need to produce compounds that analyze the carbon source. In the case of olive oil, the bacterium work to produce biosurfactant for the purpose of analysis of fatty substances and make them more polar, which in turn enter into the synthesis of secondary metabolic products and energy production as well as increasing the area of adhesion of bacteria with olive oil in the medium and this is another benefit (Swadi et al., 2013). The results of the emulsification coefficient test showed that the production medium containing olive oil as a carbon source had a high emulsification coefficient (88%), while the isolation using glycerol as a carbon source showed a low emulsification coefficient (33%). This test is the most reliable in the quantification of active substances dissolved in the medium (Ainon et al., 2013).

**Biosurfactant extraction from *Streptomyces rochei* 19:** The direct method described by (Shubhrasekhar et al., 2013) is to extract biosurfactants from isolation *Streptomyces rochei* 19. The direct method was efficient in extraction, easy and fast, and does not require much effort.

**The antibacterial activity of biosurfactant against *Staphylococcus aureus* and *Pseudomonas aeruginosa***: The biosurfactant extracted from *Streptomyces rochei* 19 showed inhibitory activity against a strain of *Pseudomonas aeruginosa* and *Staphylococcus aureus* as shown in Figure (3).

Since the biosurfactant was effective against gram-negative bacteria and gram-positive bacteria, this is probably because the extracted biosurfactant was from a broad-range type.

**Assessment of toxicity using the germination test:** The germination indexes obtained for seeds of cabbage show higher germination of about 59, 73.5, and 75.5%, with good correlation between average elongation of root and germination index. The biosurfactant produced by *Streptomyces rochei* 19 promoted no toxicity to the seeds of *Brassica oleracea* at concentrations of 1.5 and 50 mg/ml. This result is similar to those of Silva and his colleagues 2010. He shows that the biosurfactant by *Pseudomonas aeruginosa* UCP992 has no toxicity to the seeds of *Lactuca sativa* L. and *Brassica oleracea.*

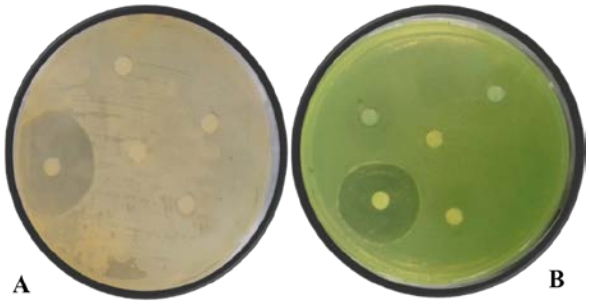


**Figure: (3).** The antimicrobial activity of biosurfactant isolated from *Streptomyces rochei* 19 on
A-*Staphylococcus aureus*
B-*Pseudomonas aruginosa*

**Thin layer chromatography (TLC):** The separation and diagnosis of biosurfactants extracted from strain 19 were detected at a flow rate of 0.9 obtained in a reddish brown spot by using ninhydrin to detect free amino groups and a brown spot by using ioden vapor, thus indicating that the biosurfactant is lipopeptide (Bezza & Chirwa, 2015).

**Gas chromatography and mass spectrometric analysis:** In GC-MS analysis 21 compounds were identified, five of them were bioactive compounds from *Streptomyces rochei* 19 strain, as shown in table (5).

**Table: (5).** Chemical constituents of chloroform-methanol extract of *Streptomyces rochei*19 identified by gas chromatography-mass spectrometer (GC/MS) analysis.

| Compounds | RT | Compound formula | Molecular weight | Activity | References |
|---|---|---|---|---|---|
| Octanoic acid | 11.147 | $C_8H_{16}O_2$ | 144 | Antimicrobial activity | Hismiogullari et ) (al., 2008 |
| Hexanoic acid | 8.048 | $C_6H_{12}O_2$ | 116 | Antimicrobial activity | Hismiogullari et ) (al., 2008 |
| Phenol, 2,4-bis(1,1-dimethylethyl)- | 15.863 | $C_{14}H_{22}O$ | 206 | Antibacterial activity | (Lata, 2015) |
| Undecanoic acid | 12.557 | $C_{11}H_{22}O_2$ | 186 | Antifungal activity | (Peres et al., 2011) |
| Dodecane, 2,6,10- trimethyl- | 12.824 | $C_{15}H_{32}$ | 212 | Antibacterial activity | Nahid et al., ) (2012 |

## CONCLUSIONS

The biosurfactant extracted from *Streptomyces rochei* 19 showed inhibitory activity against Multi-drug resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa*. In addition, the biosurfactant production from *Streptomyces rochei* 19 was non-toxic to the potential growth of *Brassica oleracea* seeds at 1.5 and 50 mg/cm$^3$. Biosurfactants were diagnosed as Lipopeptides by using thin layer chromatography and GC-Mass technique.

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# تأثير منشطات السطح الحيوية للنوع *Streptomyces rochei* على الممرضات *Staphylococcus aureus* و *Pseudomonas aeruginosa*

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**المستخلص** : جمعت *(50)* عينة من ترب ملوثة، وغير ملوثة بالهيدروكاربونات. شخصت ست عزلات تابعة للنوع *Streptomyces rochei*، *8%* من الترب الملوثة بالهيدروكاربونات، و *6%* من الترب غير الملوثة بالاعتماد على دراسة *16s* rDNA مقارنة مع المركز الوطني لمعلومات التقنية الحيوية. جمعت (*28*) عينة ممرضة لجروح و(12) لحروق عزلت منها عزلات تعود إلى النوع *Staphylococcus aureus* وبنسبة *(35.7%)*، والنوع *Pseudomonas aeruginosa* وبنسبة *16.6%*. أظهرت العزلات التابعة للنوع *Staphylococcus aureus* و *Pseudomonas aeruginosa* مقاومة متعددة للمضادات الحيوية Oxacillin، Erythromycin، Nalidixic acid و Tetracycline . أظهرت العزلة *Streptomyces rochei 19* قابلية على إنتاج المواد الحيوية الفعالة سطحياً، والتي لها فعالية مضادة ضد النوعين الممرضين *Staphylococcus aureus* و *Pseudomonas aeruginosa*. بالإضافة إلى ذلك، كان إنتاج منشطات السطح الحيوية من Streptomyces *rochei 19* غير سام للنمو المحتمل لبذور Brassica oleracea عند 1،5 و50 مجم/سم$^3$. شخصت هذه المواد كدهون ببتيدية بالاعتماد على تقنية كروماتوكرافيا الطبقة الرقيقة TLC وتقنية GC-Mass.

**الكلمات المفتاحية** : ستربتوميس; مضاد للجراثيم ; التوتر السطحي الحيوي; تقنية قياس الكتلة–الغاز.

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