CUKUROVA UNIVERSITY
INSTITUTE OF NATURAL AND APPLIED SCIENCES

MSc THESIS

Maha SALAH MOHAMED ABDELWARETH

INVESTIGATION OF THE PRESENCE OF BIOSURFACTANT PRODUCING MICROORGANISMS IN EXTREME SOILS

DEPARTMENT OF BIOLOGY

ADANA-2021

ABSTRACT

MSc THESIS

INVESTIGATION OF THE PRESENCE OF BIOSURFACTANT PRODUCING MICROORGANISMS IN EXTREME SOILS

Maha SALAH MOHAMED ABDELWARETH

CUKUROVA UNIVERSITY
INSTITUTE OF NATURAL AND APPLIED SCIENCES
DEPARTMENT OF BIOLOGY

Supervisor : Prof .Dr. Sadık DİNÇER
Year:2021, Pages: 187
Jury : Prof .Dr. Sadık DİNÇER
: Prof.Dr.Gökhan CORAL
: Asst. Prof. Dr. Burak AY

In this study, biosurfactant production was carried out by *Bacillus velezensis, Bacillus haynesii, Bacillus halotolerans, Pseudomonas reinekei, Microbacterium arborescens, Pseudomonas veronii* and *Acinetobacter lwoffii* using olive oil as a carbon source. Optimization of pH, temperature and carbon source for biosurfactant production was carried out. Antimicrobial effects of *B.velezensis, P.reinekei and M.arborescens* bacteria (50 and 100 microliters) on some pathogens (*Staphylococcus aureus ATCC 29213, Escherichia coli, Salmonella spp., Klebsiella spp. and Candida albicans*) were determined by using disc diffusion method.

According to the results obtained, the highest biosurfactant production of the isolates (*B.velezensis, P.veronii P.reinekei*) was found according to the oil spread technique after 15 days of incubation, but the highest biosurfactant production in 72 hours of incubation was in *B.halotolerans* and *B. haynesii*.

In the emulsification index measurement of the biosurfactants produced by the isolates, xylene and sunflower oil hydrocarbons reduce the surface tension by forming emulsion layers, but in the analysis with n-heptane, there is little or no emulsion layers. all biosurfactants produced by the isolates (at 50 µL and 100 µL concentrations) have inhibitory activity against the pathogens used.

As a result, maximum biosurfactant production was achieved by adding 5% olive oil as a carbon source, the medium was adjusted to pH 8 and were incubated at 37ºC. It has been determined that the produced biosurfactants reduce the surface tension and have antibacterial and antifungal effects against many pathogens.

Keywords: Biosurfactants, Bacteria, Oil displacement, Emulsification index, Antimicrobial effect, Fermentation, Haemolytic capacity, Surface tension.

ÖZ

YÜKSEK LİSANS TEZİ

EKSTREM TOPRAK ÖRNEKLERİNDEN İZOLE EDİLEN MİKROORGANİZMALARIN BİYOSÜRFEKTAN ÜRETİMLERİNİN ARAŞTIRILMASI

Maha SALAH MOHAMED ABDELWARETH

ÇUKUROVA ÜNİVERSİTESİ
FEN BİLİMLERİ ENSTİTÜSÜ
BİYOLOJİ ANABİLİM DALI

Danışman : Prof .Dr. Sadık DİNÇER
Yıl:2021, sayfa: 187
Jüri : Prof .Dr. Sadık DİNÇER
: Prof.Dr.Gökhan CORAL
: Dr.Öğr. Üyesi Burak AY

Bu çalışmada, *B.velezensis, B.haynesii, B.halotolerans, P.reinekei, M.arborescens, P.veronii* ve *A.lwoffii* tarafından karbon kaynağı olarak zeytinyağı kullanılarak biyosürfaktan üretimi gerçekleştirilmiştir. pH, sıcaklık ve karbon kaynağının biyosürfaktan üretimi için optimizasyonu gerçekleştirilmiştir. *B.velezensis, P.reinekei* ve *M.arborescens* bakterilerinin üretmiş olduğu biyosürfektanın (50 ve 100 mikrolitre), disk difüzyon yöntemi kullanılarak bazı patojenler (*S.aureus ATCC 29213, E.coli, Salmonella* spp*., Klebsiella* spp. ve *C.albicans*) üzerindeki antimikrobiyal etkileri belirlenmiştir.

Elde edilen sonuçlara göre, 15 günlük İnkübasyondan sonra yağ yayma tekniğine göre, izolatların en yüksek biyosürfaktan üretimi (*B.velezensis, P.veronii* ve *P.reinekei*) bulundu, ancak 72 saatlik inkübasyonda en yüksek biyosürfektan üretimi *B.halotolerans , B.haynesii ,M.arborescens* ve *A.lwoffii*'nin ürettiği tesbit edilmiştir .

İzolatların ürettikleri biyosürfaktanların emülsifikasyon indeksi ölçümünde ksilen ve ayçiçek yağı hidrokarbonlarının emülsiyon tabakaları oluşturarak yüzey gerilimini azalttığı ancak n-heptan ile yapılan analizde emülsiyon tabakalarının çok az olduğu veya hiç olmadığı, izolatların ürettiği tüm biyosürfaktanlar (50 µL ve 100 µL konsantrasyonlarda) kullanılan patojenlere karşı inhibitör aktiviteye sahip olduğu belirlenmiştir.

Sonuç olarak, üretim ortamına, pH 8'de, 37ºC'de ve karbon kaynağı olarak %5 zeytinyağı ilavesi ile maksimum biyosürfaktan üretimi gerçekleşmiştir. Üretilen biyosürfaktanların yüzey gerilimini azalttığı ve birçok patojene karşı antibakteriyel ve antifungal etkileri olduğu saptanmıştır.

Anahtar kelimeler: Biyosürfaktanlar, Bakteriler, Emülsifikasyon indeksi, Antimikrobiyal etki, Fermentasyon, Hemolitik kapasite, Yüzey gerilimi.

EXTENDED ABSTRACT

Pollution is recognized as one of the most critical problems facing human societies worldwide, especially in developing countries. Although caused by man and his actions, it has negative consequences for human environments and resources (Al-Bager. 2005; Anandara and Thivakaran. 2010).

As a result, pollution and its consequences are seen as one of the greatest sins of man against himself. Pollutants can cause primary damage with a direct impact on the environment or secondary damage with subtle disruptions to the delicate balance of the biological food chain that are only noticeable over long periods of time. Concerns about scarcity of raw materials, rising prices and general pollution are the biggest concerns of today's society.

The challenges of recovering and recycling waste, as well as turning waste into valuable materials, are becoming more and more important. Environmental pollution, especially heavy metal pollution, has been defined as a serious environmental problem in recent years and numerous solutions have been discovered to remove it from the environment.

Surfactant use has increased 300 percent in the United States over the past 20 years. Surfactants are now produced over 3 million tons per year worldwide. Most of these surfactants can be chemically extracted from petroleum (Banat et al. 2000). Several bacterial and fungal species produce biosurfactants, a heterogeneous group of surfactants that are secreted intracellularly or extracellularly. These naturally occurring surfactants are amphiphilic, meaning they contain both hydrophilic and hydrophobic moieties, reduce surface and interface tension with high selectivity, low toxicity, and biodegradability. Emulsification, foaming, detergent and dispersibility are all facilitated by these properties (Sneha-Chakrabarti. 2013).

Biosurfactants increase the bioavailability of hydrophobic pollutants, bind to heavy metals, have antimicrobial activity, and regulate the adhesion and

detachment of microbes to and from surfaces (Walter et al. 2010). Due to their simple chemical structure, higher stability, strong foaming ability, low toxicity, pH, environmental compatibility and biodegradability, biosurfactants outperform commercial surfactants. Biosurfactants have special functional groups that allow them to detoxify certain pollutants under conditions of high salinity, temperature and pH (Sneha-Chakrabarti. 2013).

Biosurfactants are useful in the cosmetic, pharmaceutical, oil and food industries due to their low toxicity and environmentally benign nature (Rita de Cássia et al. 2014). The use of biosurfactants for some purposes has been found to be both environmentally friendly and a cost-effective alternative to traditional complex cleaning techniques. Biosurfactants are considered as a possible option for cleaning environmental pollutants due to their diversity (Kahyaolu.1999). Environmentally friendly technologies are built on the recycling of waste materials. These chemicals are used not only as bioemulsifiers and biosurfactants, but also as antibiotics and biocontrol agents. Glycolipids, lipopeptides or lipoproteins and polymeric components make up the majority of microbial surfactants. As a result, biosurfactants and their prospective applications are attracting more attention. Surface activity, tolerance to pH, temperature and ionic strength, biodegradability, low toxicity, ability to emulsify and demulsify, and antimicrobial activity are the hallmarks of microbial surfactants.

The anticancer activity of biosurfactants was studied in mammary epithelial carcinoma cells at various concentrations. Biosurfactant has been identified as a lipopeptide type by TLC, FTIR, and GC-MS analysis. It was discovered that it has antiviral activity against shrimp white spot syndrome virus (WSSV), which significantly improves shrimp survival ($P<0.01$). The anticancer activity of biosurfactants was tested in mammary epithelial carcinoma cells at various concentrations. Among the various biosurfactant concentrations tested, 0.000 25, 0.002 5, 0.025, 0.25 and 2.5μg, 0.25 μg concentration suppressed cancer cells significantly ($P<0.05$) to 24.8%.

In this study, biosurfactant production was carried out by *B.velezensis*, *B.haynesii*, *B.halotolerans*, *P.reinekei*, *M.arborescens*, *P.veronii* and *A.lwoffii* using olive oil as a carbon source. Optimization of pH, temperature and carbon source for biosurfactant production was carried out. Antimicrobial effects of *B.velezensis*, *P.reinekei* and *M.arborescens* bacteria (50 and 100 microliters) on some pathogens (*S.aureus ATCC 29213*, *E.coli*, *Salmonella spp., Klebsiella spp.*) were determined by using disc diffusion method.

According to the results obtained, the highest biosurfactant production of the isolates (*B.velezensis*, *P.veronii* and *P.reinekei*) was found according to the oil spread technique after 15 days of incubation, but the highest biosurfactant production in 72 hours of incubation was *B.halotolerans* and *B.haynesii*.

In the emulsification index measurement of the biosurfactants produced by the isolates, xylene and sunflower oil hydrocarbons reduce the surface tension by forming emulsion layers, but in the analysis with n-heptane, there is little or no emulsion layers, all biosurfactants produced by the isolates (at 50 µL and 100 µL concentrations) have inhibitory activity against the pathogens used.

As a result, maximum biosurfactant production was achieved by adding 5% olive oil as a carbon source, the medium was adjusted to pH 8 and were incubated at 37ºC. It has been determined that the produced biosurfactants reduce the surface tension and have antibacterial and antifungal effects against many pathogens.

GENİŞLETİLMİŞ ÖZET

Kirlilik, dünya çapında, özellikle gelişmekte olan ülkelerde insan topluluklarının karşı karşıya olduğu en kritik sorunlardan biri olarak kabul edilmektedir. İnsandan ve eylemLerinden kaynaklanmasına rağmen, insan ortamLarı ve kaynakları için olumsuz sonuçları (Al-Bager. 2005; Anandara ve Thivakaran. 2010)..

Sonuç olarak, kirlilik ve sonuçları, insanın kendisine karşı en büyük günahlarından biri olarak görülmektedir. Kirleticiler, çevre üzerinde doğrudan bir etki ile birincil zarara veya biyolojik besin zincirinin hassas dengesinde yalnızca uzun süreler boyunca fark edilebilen ince bozulmalar ile ikincil hasarlara neden olabilir. Hammadde kıtlığı, artan fiyatlar ve genel kirlilik ile ilgili endişeler günümüz toplumunun en büyük çekinceleridir.

Atıkların geri kazanılması ve geri dönüştürülmesinin yanı sıra atıkların değerli malzemelere dönüştürülmesi gibi zorluklar giderek daha önemLi hale geliyor. Çevre kirliliği, özellikle ağır metal kirliliği, son yıllarda ciddi bir çevre sorunu olarak tanımLanmış ve çevreden uzaklaştırılması için sayısız çözüm keşfedilmiştir.

Sürfaktan kullanımı son 20 yılda Amerika Birleşik Devletleri'nde yüzde 300 arttı. Yüzey aktif maddeler artık dünya çapında yılda 3 milyon tondan fazla üretiliyor. Bu yüzey aktif maddelerin çoğu petrolden kimyasal olarak ekstrakte edilebilmektedir (Banat ve ark. 2000). Birkaç bakteri ve mantar türü, intraselüler veya hücre dışı olarak salgılanan heterojen bir yüzey aktif molekül grubu olan biyosürfaktanları üretirler. Bu doğal olarak mevcut yüzey aktif bileşikler amfifiliktir, yani hem hidrofilik hem de hidrofobik kısımLar içerirler, yüksek seçicilik, düşük toksisite ve biyolojik olarak parçalanabilirlik ile yüzey ve arayüz gerilimini azaltırlar. Emülsifikasyon, köpürme, deterjan ve dağılabilirliğin tümü bu özelliklerle kolaylaşmaktadır (Sneha-Chakrabarti. 2013).

Biyosürfaktanlar, hidrofobik kirleticilerin biyoyararlanımını artırarak, ağır metallere bağlanarak, antimikrobiyal aktiviteye sahip olarak ve mikropların yüzeylere yapışmasını ve yüzeylerden ayrılmasını düzenlerler (Walter ve ark. 2010). Basit kimyasal yapıları, daha yüksek stabiliteleri, güçlü köpürme kabiliyetleri, düşük toksisiteleri, pH'ları, çevresel uyumLulukları ve biyolojik olarak parçalanabilirlikleri nedeniyle biyosürfaktanlar ticari yüzey aktif maddelerden daha iyi performans gösterir. Biyosürfaktanlar, yüksek tuzluluk, sıcaklık ve pH koşulları altında belirli kirleticileri detoksifiye etmelerine izin veren özel fonksiyonel gruplara sahiptir (Sneha- Chakrabarti. 2013).

Biyosürfaktanlar, düşük toksisiteleri ve çevresel olarak iyi huylu yapıları nedeniyle kozmetik, ilaç, yağ ve gıda endüstrilerinde faydalıdır (Rita de Cássia ve ark. 2014). Bazı amaçlar için biyosürfaktanların kullanımının hem çevreye zarar vermeyen hem de geleneksel karmaşık temizleme tekniklerine karşı maliyet açısında etkin bir alternatif olduğu keşfedildi. Biyosürfaktanlar, çeşitlilikleri nedeniyle çevredeki kirleticilerin temizlenmesi için olası bir seçenek olarak değerlendirilmektedir (Kahyaolu. 1999). Çevre dostu teknolojiler, atık malzemelerin geri dönüşümü üzerine kuruludur. Bu kimyasallar sadece biyoemülgatör ve biyosürfektan olarak değil, aynı zamanda antibiyotik ve biyokontrol ajanları olarak da kullanılmaktadır. Glikolipidler, lipopeptitler veya lipoproteinler ve polimerik bileşenler, mikrobiyal yüzey aktif maddelerin çoğunluğunu oluşturur. Sonuç olarak, biyosürfaktanlar ve bunların ileriye dönük uygulamaları daha fazla ilgi görmektedir. Yüzey aktivitesi, pH, sıcaklık ve iyonik mukavemet toleransı, biyolojik olarak parçalanabilirlik, düşük toksisite, emülsiyonlaştırma ve emülsiyon giderme yeteneği ve antimikrobiyal aktivite, mikrobiyal yüzey aktif maddelerin ayırt edici özellikleridir.

Biyosürfaktanların antikanser aktivitesi, çeşitli konsantrasyonlarda meme epitelyal karsinom hücrelerinde incelenmiştir. Biyosürfaktan TLC, FTIR ve GC-MS analizi yoluyla bir lipopeptit tipi olarak tanımLanmış olup, Biyosürfaktanın, (13-Docosenamide, (Z); Mannosamin, 9– ve N,N,N',N'–tetrametil), viral

replikasyonu baskılayan ve karidesin hayatta kalmasını önemLi ölçüde artıran karides beyaz nokta sendromu virüsüne (WSSV) karşı antiviral aktiviteye sahip olduğu keşfedilmiştir (P<0.01). Biyosürfaktanların antikanser aktivitesi, çeşitli konsantrasyonlarda meme epitelyal karsinom hücrelerinde test edildi. Test edilen çeşitli biyosürfaktan konsantrasyonları arasında, 0.000 25, 0.002 5, 0.025, 0.25 ve 2.5µg, 0.25 µg konsantrasyonu kanser hücrelerini önemLi ölçüde (P<0.05) %24.8'e bastırdı.

Chandankere et al. 2014 yılında, etkili bir biyosürfaktan üreten ve hidrokarbon bozunduran bakteri suşu olan *Bacillus* methylotrophicus USTBa, tek karbon kaynağı olarak ham petrol kullanılarak hidrokarbonla kontamine sulu bir ortamdan izole edildi. pH, sıcaklık ve tuzluluk gibi büyüme parametreleri hidrokarbon bozunması için optimize edildi ve iki hafta içinde ham petrol gideriminde %90 bozunma gözlemLendi. Hidrokarbon bozunması sırasında üretilen biyosürfaktanı izlemek için yüzey gerilimi ve hücre hidrofobikliği ölçümLeri kullanıldı. 35 mg/L'lik kritik bir misel konsantrasyonu (CMC) ile üretilen biyosürfaktan, suyun yüzey gerilimini 72'den 28 mN/m'ye düşürmeyi başardı. Ham petrol üzerinde biyosürfaktan %90 emülsifikasyon aktivitesi (EI) sergiledi. Biyosürfaktanın fonksiyonel grupları, yüzey yapısı ve termostabilitesi sırasıyla nükleer manyetik rezonans spektroskopisi (NMR), X-ışını kırınımı (XRD) ve termal gravimetrik (TG) analizi ile ortaya çıkarılmıştır. USTB suşu, gaz kromatografi analizine göre, ham petrolden çeşitli alkanları verimLi bir şekilde bozundurdu. Biyosürfaktan, çeşitli sebzeler üzerinde engelleyici bir etkiye sahip değildi, ancak gram pozitif ve gram negatif bakterilere karşı güçlü antibiyotik aktivitesi vardı. Çalışma, USTBa biyosürfaktanının ham petrol kirleticilerinin biyoremediasyonu için uygun bir aday olarak kullanılmasını önermektedir.

Bu çalışmada, *B.velezensis*, *B.haynesii*, *B.halotolerans*, *P.reinekei*, *M.arborescens*, *P.veronii* and *A.lwoffii* tarafından karbon kaynağı olarak zeytinyağı kullanılarak biyosürfaktan üretimi gerçekleştirilmiştir. pH, sıcaklık ve karbon kaynağının biyosürfaktan üretimi için optimizasyonu gerçekleştirilmiştir.

B.velezensis, *P.reinekei* ve *M.arborescens* bakterilerinin üretmiş olduğu biyosürfektanın (50 ve 100 mikrolitre), disk difüzyon yöntemi kullanılarak bazı patojenler (*S.aureus ATCC 29213*, *E.coli* , *Salmonella spp*., *Klebsiella spp*. ve *C.albicans*) üzerindeki antimikrobiyal etkileri belirlenmiştir.

Elde edilen sonuçlara göre, 15 günlük İnkübasyondan sonra yağ yayma tekniğine göre, izolatların en yüksek biyosürfaktan üretimi (*B.velezensis*, *P.veronii* ve *P.reinekei*) bulundu, ancak 72 saatlik inkübasyonda en yüksek biyosürfektan üretimi *B.halotolerans*, *B.haynesii*, *M.arborescens* ve *A.lwoffii*'nin ürettiği tesbit edilmiştir .

İzolatların ürettikleri biyosürfaktanların emülsifikasyon indeksi ölçümünde ksilen ve ayçiçek yağı hidrokarbonlarının emülsiyon tabakaları oluşturarak yüzey gerilimini azalttığı ancak n-heptan ile yapılan analizde emülsiyon tabakalarının çok az olduğu veya hiç olmadığı, izolatların ürettiği tüm biyosürfaktanlar (50 µL ve 100 µL konsantrasyonlarda) kullanılan patojenlere karşı inhibitör aktiviteye sahip olduğu belirlenmiştir.

Sonuç olarak, üretim ortamına, pH 8'de, 37ºC'de ve karbon kaynağı olarak %5 zeytinyağı ilavesi ile maksimum biyosürfaktan üretimi gerçekleşmiştir. Üretilen biyosürfaktanların yüzey gerilimini azalttığı ve birçok patojene karşı antibakteriyel ve antifungal etkileri olduğu saptanmıştır.

ACKNOWLEDGMENT

All gratitude is due to almighty ALLAH who guided me to prepare this thesis successfully. I would like to express my deepest appreciation to Prof. Dr. Sadık DİNÇER Professor of Microbiology, Biology Department, Faculty of Science and Letters, Cukurova University, for his supervision, unfailing guidance, valuable discussions, remarks, advice, assistance and various facilities.

I am greatly indebted to Res. Asst. Fatıma Masume Uslu, Biology Department, Faculty of Science and Letters, Cukurova University, for her sincere supervision, guidance and aid in laboratory, fruitful discussions and continuous help during progress of the work.

I would like to express my deep thanks to the Department of Biology, for offering the required facilities and supporting me during my steps of carrying out this research, and Cukurova University for their financial support. All gratitude to the Research Fund.

I would like to thank the Inorganic Chemistry Laboratory in the Faculty of Science and Letters, Cukurova University, for the help with the FTIR analysis.

I would like to thank all the staff and assisting staff members of Biology Department, Faculty of Science and Letters, Cukurova University, for their sincere cooperation, all the thanks to my very valuable teachers, whose support and interest I have always seen at every stage of my work and life.

I want to express my appreciation to my friends in the Bacteriology lab who helped and supported me in my Research. All the thanks and the love to my close friends who have an important place in my life, especially Mr. Mohamed Alaa all the thanks for his continuous support and for his help in the statistical analysis and many thanks for Ms Noha Kamel for her continuous support and help.

I'm highly grateful to my parents for their endless encouragement, loving, precious advice and prayers that enabled me to pursue knowledge and education. I would to offer my deepest thanks to my sisters and all the members of my family for their help, assistance, moral support and encouragement.

CONTENTS **PAGES**

LIST OF TABLES **PAGES**

LIST OF FIGURES **PAGES**

ICONS AND ABBREVIATIONS

α	: Alpha
β	: Beta
atm	: atmosphere
ATP	: Adenosine Triphosphate
E	: Emulsification index
EDTA	: Ethylenediaminetetraacetic acid
EC	: Enzyme Commission
FTIR	: Fourier Transform Infrared Spectroscopy
g	: Number of revolutions per minute
HPLC	: High Performance Liquid Chromatography
IR	: Infrared
IUBMB	: International Union of Biochemistry and Molecular Biology
MEOR	: Microbial Enhanced Oil Recovery
mN/m	: Milli Newton per meter
NMR	: NMR spectroscopy
OD	: Optical Density
PAGE	: Polyacrylamide Gel Electrophoresis
rpm	: revolutions per minute
sp.	: Species (genre, singular)
spp.	: Species (genre, plural)
h/h	: Volume/Volume
a/h	: Weight/Volume
a/a	: Weight/Weight

1. INTRODUCTION

Environmental pollution is not a new occurrence, but it remains the world's most serious problem and one of the leading causes of illness and mortality. Human activities such as urbanization, industrialization, mining, and exploration all contribute to global environmental pollution. This burden is shared by both developed and developing countries, with developed countries doing a better job of environmental preservation due to increased awareness and tougher legislation. Despite the fact that pollution has received worldwide attention, the consequences of its severe long-term consequences are still felt. Raw material shortages, rising prices, and general pollution have all been major concerns in recent years. As a result, issues such as waste recovery and recycling, as well as the conversion of trash into valuable products, are becoming more important. Environmental pollution, particularly heavy metal contamination, is a major problem in today's society, and many solutions are being developed to address it. In the last 20 years, the use of surfactants has increased by 300 percent in the United States. Surfactants are now produced at a rate of more than 3 million tons per year all over the world. Chemically, the majority of these surfactants can be made from petroleum. Banat et al. (Banat et al. 2000).

Heavy metals are naturally occurring contaminants in the earth's crust that cannot be degraded or removed, making them long-lasting environmental contaminants. They enter the body through food, air, and water, where they build up over time. They exist as ores in rocks of various chemical compositions, from which minerals are extracted. Iron, arsenic, lead, lead-zinc, cobalt, gold-silver, and nickel sulphides, as well as oxides such as aluminum, manganese, gold, selenium, and antimony, are sulphides. Heavy metal pollution is particularly common in mining areas and abandoned mine sites, and contamination decreases as one moves away from the mining area (Peplow. 1999). Acid water leaches these metals out and transports them downstream or to the sea. As a result of mining activity, water

bodies are egregiously polluted (Garbarino et al. 1995). The negative effects of heavy metals on the body when taken in excess of bio-recommended levels are referred to as heavy metals' bio toxic effects. GI disorders, diarrhoea, stomatitis, tremor, haemoglobinuria causing a rust–red color to stool, ataxia, paralysis, vomiting and convulsion, depression, and pneumonitis have all been reported as general signs associated with cadmium, lead, arsenic, mercury, zinc, copper, and aluminum poisoning (Mccluggage. 1991). Toxic (acute, sub-chronic, or chronic), neurotoxic, mutagenic, carcinogenic, or teratogenic effects are possible

Table 1.1. Potential industrial and agricultural sources for heavy metals in the environment.

Metal	Sources
Fe	Pigments and paints; fuel; refineries; textile.
Mn & Zn	Batteries and electrical; pigments and paints; alloys and solders; pesticides; glass; fertilizers; refiners; fuel.
Pb	Batteries and electrical; pigments and paints; alloys and solders; pesticides; glass; fertilizer; refiners; fuel; plastic.
Cd	Batteries and electrical; pigments and paints; alloys and solids; fuel; plastic; fertilizers.
Ni	Batteries and electrical; pigments and paints; alloys and solids; fuel; catalysts; fertilizers.
Cu	Batteries and electrical; pigments and paints; alloys and solid; fuel; catalysts; fertilizers; pesticides.
Cr	Pigments; fertilizers; textile.

1.1. Environmental Pollution By Heavy Metals In Egypt

1.1.1. Egypt's Delta And Mediterranean Coast

The River Nile gets wastewater discharge from 124-point sources between Aswan and the El-Kanater Barrage, with 67 of them being agricultural drains and the rest being industrial sources. The entire volume of wastewater discharged into the Nile's main stem each year is estimated to be 2628 million cubic meters, with waste water accounting for 15% (Zaki et all. 2014).

1.1.2. Egypt's Red Sea

The Aqaba Gulf is a vast semi-closed Red Sea basin. Egypt, Israel, Jordan, and Saudi Arabia all have Gulf beaches. Nowadays, urban and industrial pollutants (Walker and Ormond. 1982), shipping and port activity (Badran and Foster. 1998), and tourism (Hawkins and Roberts. 1994) all have a significant impact on the Gulf. Furthermore, land-based industries such as clinker manufacturing and fertilizer manufacturing, as well as sea–water desalinization in Eilat, may have an impact on the prevalence of heavy metals. The concentrations of several contaminants in the Aqaba Gulf region increased as a result of these anthropogenic activities.

The distribution and concentration of heavy metals (Fe, Mn, Zn, Ni, Co, Cr, Cu, and Cd) in the sediments of the Egyptian shore of the Aqaba Gulf have been studied (Youssef and El Said. 2011). Large heavy metals fluctuations were discovered in the obtained results. Furthermore, highly significant relationships were found between Fe, Cu, Ni, and Co heavy metals and their similar lithogenic origins, as well as their input sources. Mn (in Lake Edku) and Cd (in Lake Manzala) were found to be higher than the sediment quality recommendations in sediment samples obtained from northern Delta Lakes (Edku, Borollus, and Manzala). During May 2010, surface sediment samples were collected from an Egyptian Mediterranean beach to investigate the influence of land-based activity on Hg and Sn distribution (Hamed et all. 2013). Sinai Beach had the greatest mean concentration of Hg in sediments (14.938 ng/g), while Alexandria Beach had the greatest mean value of Sn (1.414 lg/g). (Omar. 2013).

1.2. Biosurfactant

Biosurfactanates are a heterogeneous group of compounds, surface-active particles that are released extracellularly or adjacent to the cell membrane by different types of bacteria and fungi (Anandara and Thivakaran. 2010). Biosurfactanates are naturally amphiphilic in nature, contain hydrophilic and hydrophobic moieties that reduce the surface and interfacial tension with

biodegradability, high specificity and low toxicity. This expedite properties such as foaming, emulsification, dispersing and detergency. (Sneha-Chakrabarti. 2013). There are two types of biosurfactants high molecular weight biosurfactants that contain polysaccharides and proteins, and low molecular weight biosurfactants which contain glycolipids and lipopeptides. According to their charges, they can be classified as anionic, non-ionic and cationic biosurfactants (Varjani et all. 2014). This promotes microbial growth by increasing the availability of hydrophobic nutrients and converting them into harmless products. Due to the unique feature of reducing surface tension, the area of contact of insoluble compounds increases, as well as the mobility, bioavailability and biodegradation of hydrophobic pollutants. Biosurfactants have proven to be very useful for their producing strains by improving the bioavailability of hydrophobic contaminants, connect to heavy metals, own antimicrobial activity and control the attachment or detachment of microorganisms to and from surfaces. Biosurfactants are preferred over chemical surfactants with its simple chemical structure, increased stability, high foaming ability, low toxicity, pH, biodegradability and environmental compatibility. Biosurfactants possess specific functional groups which provide specificity in detoxification of certain pollutants in extreme salinity, temperature, and pH. Their properties like low toxicity and eco-friendly nature support the applicability of biosurfactants in cosmetic, pharmaceutical, oil and food industries (Rita de Cássia et all. 2014).

The use of petroleum and petroleum-based polymers has gone up significantly. Although these polymers can be beneficial in a variety of ways, their accumulation in non-biological degradation and contamination has been a source of concern. Water is the main by-product associated with oil production and gas exploration, so the field produces a lot of it (Igunnu et al. 2012). Water from oil fields contains a lot of hydrocarbons, including petroleum, diesel oils, dispersed oil, a lot of aromatic compounds, and a lot of radionuclides. The water produced contains specific indigenous hydrocarbon-decomposing microorganisms that can

use toxic waste products from their metabolism during bioremediation to improve their survival. Bacteria that decompose hydrocarbons were discovered to be the most active. Biosurfactants (BS) are surface-active heterogeneous amphiphilic compounds that lower surface and interfacial tension. By lowering surface tension and simplifying hydrocarbon uptake through hydrocarbon solubilization, biosurfactants aid cell survival and promote growth kinetics. Biosurfactant producers are a type of hydrocarbon degrader with a high surface activity that aids in the breakdown of harmful metabolites. Biosurfactants' surface activity contributes to the formation of microemulsions, in which hydrocarbon solubility can occur (Tabatabaee et al. 2005; Ahmad et al. 2016) discovered that bacteria from the *Pseudomonas*, *Bacillus*, *Klebsiella spp*., Baumannii, Corynebacterium, *Archromobacterium, Falvobacterium*, and Gamma species produce effective biosurfactants. The majority of biosurfactants are made from immiscible hydrocarbons, but some are made from miscible hydrocarbons as well. Hydrophobic and hydrophilic moieties make up biosurfactants. Hydrophilic moieties help to lower surface tension and promote hydrocarbon desorption and dissolution. The interactions between surfactant molecules and liquid droplets are mediated by hydrophobic moieties (Pacwa-Pociniczak et al. 2011). Biosurfactants are divided into two types: low-molecular-weight biosurfactants, which are primarily used to reduce surface and interfacial tension, and high-molecular biosurfactants, which are used for emulsification and stabilization. Biosurfactants' chemical composition is determined by the biosynthetic pathway and enzymes (primarily transferases and synthases) required for their production. Biosurfactants are classified as lipopeptides, glycolipids, phospholipids, and neutral lipids based on their chemical composition. Polymeric biosurfactants, such as biodispersan and emulsions, are also available (Batool et al. 2017). Some of the most well-studied biosurfactants include rhamnolipids, Sophorolipids, lipomanan, iturin, emulsan, liposen, surfactin, lichenysin, alasan, and others. Rhamnolipids (produced by *Pseudomonas* aeruginosa) and surfactants (produced by *Bacillus subtilis*) are the

two most common bacterial biosurfactants. Glycolipids, particularly rhamnolipids, are studied in depth among all of these classes. Rhamnolipids are important in part because of their high biological activity and antibacterial properties. Biosurfactant production and synthesis are affected by a variety of external factors, including pH, temperature, carbon and nitrogen sources, and so on. At 37^{o} C and pH 7, maximum growth was observed using glucose. The RhlA enzyme is responsible for the start of rhamnolipid biosynthesis.

The rhlC and rhlB genes encode an enzyme necessary for the synthesis of rhamnolipids (Pantazaki et all. 2011). The rmLA, rmLB, rmLC, and rmLD genes are essential for the synthesis of rhamnose skeleton. Recent developments in this field show that biosurfactants are an important part of the growth and reproduction of most producers of biosurfactants, for example, *Bacillus* needs surfactin (biosurfactant) for formation of fruiting body it needs for reproduction (Ebrahimi et all. 2012). Some of the biosurfactants are found on the cell membrane, but most of them are released into the extracellular environment, where they help the cell survive in various biophysiochemical ways. Biosurfactants differ from their synthetic counterparts in their biodegradability, low toxicity low irritancy and ease of synthesis and extraction. Although the most important use of biosurfactants, until today, in the petroleum and biodiesel industries in the fields of oil purification, oil quality improvement, and removal of deposits from oil reservoirs, biosurfactants also have various applications in the environmental sciences. These biosurfactants, due to their ability to decompose hydrocarbons and their biodegradable nature, have high potential for environmental bioremediation of oil-contaminated sites, soil contamination with heavy metals and pesticides, flushing and cleaning of oil reservoirs, etc (Yadav et all. 2016). This feature of biosurfactants can also be used for management of wastewater pollution (Batool et all. 2017). Emulsification and surface-active features of biosurfactants, have made their use as detergents, foaming and wetting agents, flocculants, etc. possible (Hashemi et all. 2016). the high compatibility with human skin, most cosmetic

industries use biosurfactants in shampoos, tooth pastes and other products (Mahalingam and Sampath. 2014). The food industry also uses biosurfactants, such as these, that tends to preserve the quality of fat-based products. Some biosurfactants show therapeutic and antimicrobial features. Biosurfactants are used as biocontrol, for plant disease and plant protection, due to their microbicidal effect. Rhamnolipids have strong antifungal properties. Rhamnolipids are also necessary to reduce the toxicity of metals.

1.2.1. Biosurfactant Features

The special properties of biosurfactants in comparison with their chemically synthesized counterparts and the wide availability of the substrate made them suitable for commercial use. The distinguishing features of microbial surfactants are related to their surface activity, tolerance to temperature, pH and ionic strength, low toxicity, biodegradability, antimicrobial activity, emulsifying and demulsifying ability. The main distinctive characteristics of each property of biosurfactant are discussed below:

1.2.1.1. Surface And Interface Activity

Surfactant helps in reducing surface tension and the interfacial tension. Surfactant produced by B. subtilis is capable of reducing the surface tension of water to 25 mn m-1 and the interfacial tension of water/hexadecane to less than 1 mn m-1 (Cooper et al. 1981). In general, biosurfactants are more effective and their Critical Micelle Concentration (CMC) is several times lower than that of chemical surfactants, i.e., less surfactant is required to maximize surface tension reduction (Desai and Banat. 1997).

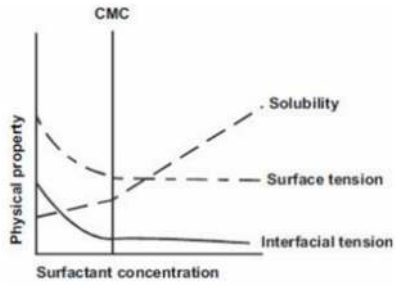


Figure 1.1. Relationship of surface tension, interfacial tension and the CMC with surfactant concentration (mulligan. 2005).

1.2.1.2. Temperature and PH Tolerance

The production of biosurfactants by extremophiles has attracted a lot of attention over the last decades, as they can be considered to have commercial significance. Most of the biosurfactants and their surface activity are resistant towards environmental factors such as temperature and pH. (McInerney et al. 1990) reported that lichenysin from *Bacillus licheniformis* was found to be resistant to pH between 4.5 and 9.0, temperature up to 50°C and NaCl and Ca concentrations up to 50 and 25 g L-1, respectively. Biosurfactant produced by Arthrobacter protophormiae, proven to be both thermostable (30-100°C) and pH (2 to 12), stable. Since, industrial processes involving exposure to extreme temperatures, pH, and pressure, it is necessary to isolate new microbial products that are able to work under these extreme conditions.

1.2.1.3. Biodegradability

Biosurfactants are appropriate for environmental applications such as bioremediation/biosorption (Mulligan et al. 2001). They can be easily degraded when compared to synthetic surfactants The increasing environmental concern made us to search for alternative products such as biosurfactants (Cameotra and Makkar. 2004). biodegradable biosurfactants from marine microorganisms were concerned for the biosorption of poorly soluble polycyclic aromatic hydrocarbon,

phenanthrene contaminated in aquatic surfaces controlled the blooms of marine algae, Cochlodinium using the biodegradable biosurfactant sophorolipid with a removal rate of up to 90% within 30 minutes of treatment.

1.2.1.4. Low Toxicity

Although there is very little literature on the toxicity of biosurfactants, are generally considered low or non-toxic products that are suitable for the pharmaceutical, cosmetic, and food industries. (Poremba et al. 1991) showed that the higher toxicity of the chemical-derived surfactant (Corexit) which showed a LC50 against Photobacterium phosphoreum that found to be 10 times lower than of rhamnolipids. (Flasz et al. 1998) indicated the biosurfactant as non-toxic and non-mutagenic when they compared the toxicity and mutagenicity profile of biosurfactant from *Pseudomonas aeruginosa* and chemically derived surfactants. The low toxicity profile of biosurfactant, sophorolipids from Candida bombicola made them beneficial in food industries.

1.2.1.5. Emulsion Forming and Emulsion Breaking

Biosurfactants can act as emulsifiers or preservatives and can be stored as a stable emulsion for months, if not years (Velikonya and Kosarich. 1993). An emulsion can be defined as a heterogeneous system consisting of one immiscible liquid, distributed in another in the form of droplets, the diameter of which usually does not exceed 0.1 mm. Emulsions are usually of two types: water in oil (o/v) or oil in water (o/v). They possess a minimal stability which can be stabilized by additives such as biosurfactants, and can be stored as a stable emulsion for months, if not years. Liposan is a water-soluble emulsifier produced by *Candida lipolytica*, which is used to emulsify edible oils, through coating droplets of oil, which leads to the formation of a stable emulsion. These liposans are widely used in the cosmetic and food industries for the production of oil-water emulsions, to create stable emulsions.

1.2.1.6. Antiadhesive Agents

A biofilm can be defined as a group of bacteria or other organic matter that have colonized or accumulated on any surface. The first step on biofilm formation is bacterial adherence over the surface was influenced by several factors, including the type of micro-organisms, the environment, the water and electric charge on the surface and the ability of micro-organisms to produce extracellular polymers that help cells stay afloat. Biosurfactants can be used to change the hydrophobicity of the surface, which, in turn, can affect the adhesion of microbes to the surface. A surfactant from Streptococcus thermophilus inhibits colonization of other thermophilic strains of Streptococcus over the steel which are responsible for contamination .in the same way, a biosurfactant from *Pseudomonas fluorescens* blocked the attachment of Listeria monocytogenes onto steel surface (Chakrabarti. 2012).

1.2.2. Biosurfactant Types

In the classification of synthetic surfactants, they depend on the ionic charge in the polar part of the molecule. Surfactants are classified according to the presence or absence of an electrical charge as anionic, cationic, non-ionic or amphoteric (Maneerat. 2005). Studies have proven that most biosurfactants are anionic or neutral, while some are cationic, such as those that contain amine groups. The hydrophobic moiety is recognized by long-chain fatty acids and the hydrophilic moiety may be an amino acid, carbohydrate, cyclic peptide, carboxyl acid, phosphate or alcohol. Biosurfactants are often classified according to their biochemical nature or the microbial producer species. With regard to the structure, these compounds are categorized into groups.

1.2.2.1. Glycolipids

Glycolipids make up the majority of known biosurfactants. They're made up of sugar and hydroxy aliphatic acids, which are long-chain aliphatic acids.

Either ether or ester gathering methods are used in the combination. Rhamnolipids, sophorolipids, and trehalolipids are the most well-known glycolipids.

1.2.2.1.(1). Rhamnolipids

Rhamnolipids are glycolipids in which one or two rhamnose molecules are linked to one or two hydroxy decanoic acid molecules. It is a widely studied that the main glycolipid is produced by *P. aeruginosa*.

1.2.2.1.(2). Sophorolipids

Sophorolipids are formed from a dimeric carbohydrate sophorose linked to a long-chain hydroxyl fatty acid by glycosidic linkage and released by yeasts. Sophorolipids are made up of at least six to nine hydrophobic sophorolipids, and the lactone form is preferred for many applications.

1.2.2.1.(3). Trehalolipids

Trehalolipids are different type of glycolipid. Disaccharide trehalose linked at C-6 and C-6 to mycolic corrosive is connected with most types of Mycobacterium, Corynebacterium and Nocardia. Mycolic acids are the long chain, α-spread and β-hydroxy unsaturated fats. Trehalolipids from various living beings change in the size and structure of mycolic corrosive, the number of carbon molecules exhibit and the degree of unsaturation. Trehalose lipids produced by *Rhodococcus erythropolis* and *Arthrobacter sp*. decreased the surface and interfacial tension in culture stock.

1.2.2.2. Surfactin

The most promising biosurfactants that is created by *Bacillus subtilis*. It is comprised of a seven aminocorrosive ring structure joined to an unsaturated fat chain by lactone linkage methods. It minimizes the surface tension from 72 to 27.9 mN/m at a constant level of 0.005%.

1.2.2.2.(1). Lipopeptides And Lipoproteins

Lipopeptides are surfactin. A very large number of cyclic lipopeptides, including lipopeptide anti-toxins (polymyxins) and decapeptide antitoxins (gramicidina)are included. These include a lipid linked to a polypeptide chain. (Singh and Cameotra. 2004) reported the antibacterial properties of the lipopeptide iturin that produced by *Bacillus subtilis*. At the same time, it was found that it is active even after autoclave sterilization, pH 5-11 and with a shelf life of 6 months at a temperature of -18°C.

1.2.2.3. Lichenysin

Some of the biosurfacants that released by *Bacillus* licheniformis are synergistic and show a high level of temperature, salt and pH tolerance. Their auxiliary and physio-synthetic properties to surfactin are also comparable. The surface tension of water to 27mN/m and interface tension between water or hexadecans to 0.36mN/m can be decreased by surfactants provided by B. licheniformis.

1.2.2.4. Fatty Acids, Phospholipids and Neutral Lipids

A few microbes and yeast manufacture enormous amounts of unsaturated fats and Phospholipid surfactants by the development of n-alkanes.1-N, phosphatidyl ethanolamine rich vesicles that optically form smaller alkane emulsions in water are released in Acinetobacter spp. These biosurfactant are fundamental for therapeutic use. (Gautam and Tyagi.2006) found that the phospholipid protein deficient complex in prematurely-born children was the main cause for breathing failure. They further indicated that the genes responsible for the surfactant can be isolated and cloned for fermentation.

1.2.2.5. Polymeric Biosurfactants

They minimized the interfacial tension between immiscible liquids and form stable emulsions. The best anticipated polymeric biosurfactants are alasan, liposan, lipomanan emulsan and some other polysaccharid proteins. an extracellular strong polyanionic amphipathics heteropolysaccharide bioemulsifier is produced by *Acinetobacter calcoaceticus* RAG-1. Emulsan is a powerful emisifying specialist for hydrocarbons in water, even at a fixation as low as 0.001 to 0.01%. Liposan is an extracellular water-soluble emulsifier that released by *Candida lipolytica* and comprised of 83% carbohydrate and 17% protein.

1.2.2.6. Particulate Biosurfactants

Extracellular film vesicles segment hydrocarbons to compose a micro-emulsion, that plays a major role in alkane take-up by microbial cells. as an example, Vesicles of *Acinetobacter sp*. which have a distance across of 20-50 nm and a light thickness of 1.158 cg/cm3, they are rich in phospholipids and lipopolysaccharides, showing good emulsification activity (Kim et all. 1997).

Table 1.2. Some of the common biosurfactants with their producing microorganisms.

Organisms	Biosurfactants	Sources	References
Pseudomonas aeruginosa	Rhamnolipids	Petrochemical wastewater, soap stock, Petroleum contaminated soil, etc.	Whang, et al., 2008; Haba, et al., 2003; Wei, et al., 2008.
Pseudoxanthomonas spp PNK-04			Nayak, et al., 2009.
Pseudomonas alcaligenes			Oliveira, et al., 2009.
Rhodococcus erythropolis 51T7 *Micrococcus luteus* BN56	Trehalose lipids	Oil contaminated soil, Hydrocarbon gas station soil, etc.	Marquez, et al., 2009; Tuleva, et al., 2009.
Bacillus subtilis ATCC 21332	Surfactin	Petroleum sludge, Waste soybean oil, etc.	Pornsunthorntawee, et al., 2008; Lee, et al., 2008.
Candida antarctica	Mannosylerythritol lipids	Vegetable oil and soybean oil waste	Kim, et al., 2002; Kitamoto, et al., 2001.
Gordonia spp BS29	Bioemulsan	Diesel contaminated soil	Franzetti, et al., 2009.

1.2.3. Sources of Biosurfactant (Producer Microorganisms)

Biosurfactants with various molecular structures can be produced by a wide range of microorganisms. Microorganisms utilise a variety of organic substances as a source of carbon and energy in order to grow. When a carbon source is in an insoluble form, such as a hydrocarbon, microorganisms produce biosurfactants, like rhamnolipids produced by various *Pseudomonas spp*. and sophorolipids produced by several *Torulopsis spp*. Other microbes have the ability to alter the structure of their cell walls by creating nonionic or lipopolysaccharides surfactants in their cell walls. *Rhodococcus erythropolis, Mycobacterium spp.*, and *Arthrobacter spp*. are examples of this category. Biosurfactant-producing bacteria from the genera *Pseudomonas* and *Bacillus* have been described in the literature as large producers of biosurfactants. Rhamnolipids that released by *Pseudomonas aeruginosa* have been investigated (Abdel-Mawgoud et al. 2010; Chrzanowski et al. 2012; Henkel et al. 2012). The sort of fermenter, pH, nutrients, substrates, and temperatures used all affect the composition and yield. Surfactin, a lipopeptide produced by *Bacillus subtilis*, has seven amino acids connected to carboxyl and hydroxyl groups of C14 acid. Surfactin concentrations of less than 0.005% reduce surface tension to 27 mN/m, putting it as one of the most powerful biosurfactants. Surfactin's solubility and surfactant capacity, on the other hand, are dependent on the type of substrate. While the topic of bacterial biosurfactant production is well-studied, fungi are largely unknown producers of biosurfactants. *Candida spp., Aspergillus ustus*, and *Trichosporon ashii* are the most studied fungus. *Candida* species have been successfully used in the fermentation of hydrocarbons and subsequent production of biosurfactants. (Table1.2) summarizes the major biosurfactant classes and their associated producer microorganisms.

1.2.4. Chemical Structure for Some Biosurfactants

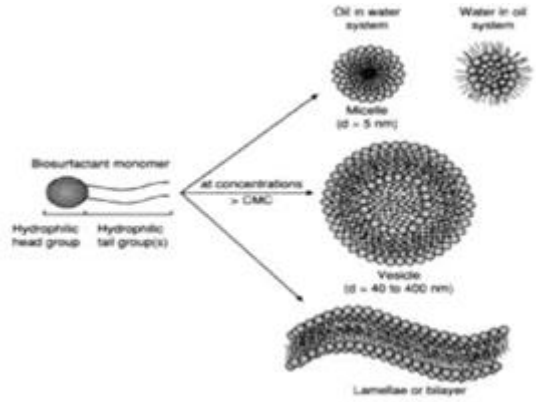


Figure 1.2. Biosurfactant molecular structure (Bodour and Maier. 2012)

According to their physical structures, surfactants obtained by chemical means are generally classified according to the polar groups in their structures. The hydrophilic group is often called the head group and has a highly polar or charge characteristic. Anionic surfactants include conventionally produced soaps, synthetic detergents, sulfonates and sulfates. Cationic surfactants are usually quaternary ammonium, imidazolium or alkyl pyridinium compounds. Zwitterionic surfactants (amphoteric) usually have betaine and sulfobetaine forms. These ingredients are used in more sensitive skin than anionic surfactants and especially in baby shampoos and toilet products due to their low eye-eye relief. Nonionic surfactants are always suppressed by ethoxylates. Surfactants in this class include some semipolar compounds such as amineoxides, sulfoxides and phospholinoxides. In combination, biosurfactants contain both nonionic and anionic groups such as alkyl ethoxy sulfates. They are surfactants that can come into direct contact with the skin, such as toothpastes and shampoos. Finally, the Hydrophobic Groups refers to a simple hydrocarbon group, often called the tail (end) part.

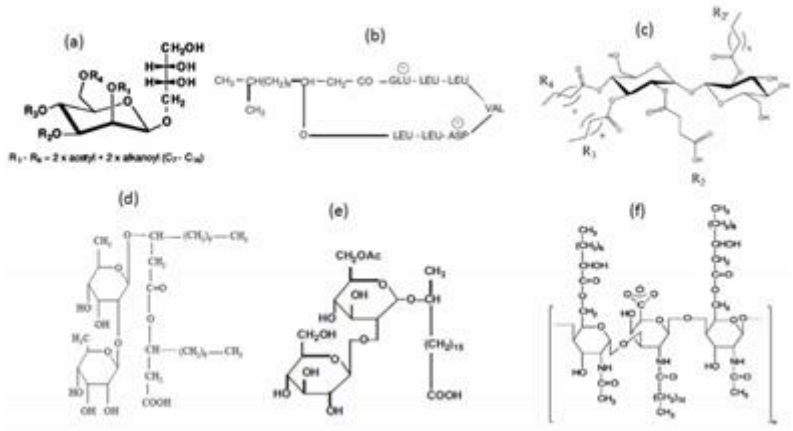


Figure 1.3. Some of the common biosurfactants molecular structures (Uyar and Yalcin. 2013)

1.2.5. Application Of Biosurfactants

Biosurfactants have use in a variety of industrial processes, as well as potential new uses in the future, due to their structures and qualities. Biosurfactants are projected to be dubbed the "multifunctional materials" of the twenty-first century (Marchant and Banat. 2012).

1.2.5.1. Environmental Applications

Because biosurfactants can be used in oil spill cleanup, oil residue removal from storage tanks, microbial-enhanced oil recovery, and soil and water bioremediation, the petroleum sector is now the largest market for these components (Gautam and Tyagi. 2006; Singh et al. 2007). The unintentional or deliberate release of organic or inorganic compounds into the environment is a common cause of environmental contamination caused by industrial activities, which poses a problem for remediation because these compounds easily bond to soil particles and spread in an aqueous medium. The goal of using biosurfactants in the decontamination of organic substances such as hydrocarbons is to increase their bioavailability or mobilize and remove contaminants during clean-up through

pseudo-solubilization and emulsification. Using biosurfactants to recover inorganic compounds such as heavy metals, on the other hand, necessitates chelation and the removal of these ions during the washing phase, which is aided by chemical interactions between amphipathic molecules and metal ions.

1.2.5.2. Application Of Biosurfactants In Microbial-Enhanced Oil Recovery

Microbially enhanced oil recovery can also benefit from biosurfactants (MEOR). MEOR technologies are utilized to recover oil that remains in reservoirs primary (mechanical) and secondary (physical) recovery (Singh et al. 2007; Packwa-Pociniczak et al. 2011). Enhanced oil recovery is a tertiary process that uses microorganisms or their metabolites, such as biosurfactants, biomass, biopolymers, acids, gases, solvents and enzymes, to extract more oil from drained deposits. One of the most promising approaches for recovering a significant part of residual oil is to use biosurfactants in MEOR. The leftover oil is frequently found in difficult-to-reach areas of the reservoir, where it is trapped in pores by capillary pressure. Biosurfactants lower the interfacial tensions between oil and water and oil and rock, lowering the capillary pressures that prevent oil from flowing through the pores of the rock. Biosurfactants can also produce an emulsion by forming a strong bond with the oil/water interface, stabilizing the oil desorbed in the water and allowing it to be removed with the injection of water (Marchant and Banat. 2012).

1.2.5.3. Application of Biosurfactants in Bioremediation

The ability of living organisms to transform or mineralize organic pollutants in order to produce less toxic compounds that may be assimilated into the natural biogeochemical cycle is referred to as biorremediation. However, factors such as oxygen, pH, the availability of macro and micronutrients, the physicochemical features of the contaminant's pollution history, and particles to which organisms and contaminants may be adsorbed all influence the biodegradability of these contaminants (Margesin and Schinner. 2001). These

events can either increase the bioavailability of soluble pollutants, resulting in a faster rate of biodegradation, or hinder biodegradation. Despite the various successful applications of biosurfactants, the literature shows contradictory bioremediation findings. Rhamnolipids from P. aeruginosa, for example, induce the breakdown of n hexadecane, whereas those from the Rhodococcus genus do not, demonstrating the specificity of microorganisms. However, a biosurfactant from R. erythropolis 3C-9 was discovered to significantly accelerate the rate of n-hexadecane breakdown. As a result, the impact of a biosurfactant on bioremediation processes is unexpected, and its efficacy must be determined experimentally (Franzetti et al. 2008). Bioremediation's mechanism is as follows: Biosurfactants play a role in bioremediation in at least two ways: raising the surface area of hydrophobic water-insoluble substrates and enhancing the bioavailability of hydrophobic substances. Many hydrocarbons, particularly the Polycyclic Aromatic Hydrocarbons (PAHs), have a poor water solubility, which may limit their availability to microorganisms, posing a barrier for bioremediation of contaminated areas. Surfactants were thought to increase the bioavailability of hydrophobic substances.

1.2.5.4. Application of Biosurfactants in The Removal Of Heavy Metals

Heavy metals are inorganic contaminants that occur naturally in rock, soil, plants, and animals and pose the greatest risk to human health. Metals can be found in a variety of forms, including ions dissolved in water, vapour, and minerals in rock, sand, and soil. Metals can also be trapped by air particles or linked to organic or inorganic compounds. Metals are released into the air and water by both natural and anthropogenic mechanisms. Heavy metal contamination is caused by a variety of industrial activities, including mining, metal forging, automobile battery manufacture, vehicle and industrial waste dump emissions, and ash diffusion from incineration processes (Hong et al. 2002). Heavy metals in soil can pose major difficulties because they are not biodegradable, resulting in pollution of biological

systems and the subsoil through the lixiviation process (Morrrison, 1983) Lead, for example, is reported in 15% of contaminated lands in the United States, followed by chromium, cadmium, and copper, which are found in 7–11% of soils. To cut expenses related with the treatment of heavy metal-contaminated soil, a variety of technologies have been devised and used (Peng et al. 2008). To treat polluted soil, two fundamental technologies are used. To minimize migration, the first method involves immobilizing heavy metals in a solid matrix that is strongly attached to the soil. However, because the soil cannot be reused and long-term monitoring is required, this technology is not a permanent solution to the problem. Through desorption and solubilization, the second method enhances metal mobility and migration to a liquid phase. This technology is seen as a long-term solution since it allows for the recycling of remediated soil and, as a result, land reuse (Hashim et al. 2011). The most extensively used procedures include cleansing soil with acids and chelating chemicals such as ethylenediaminetetraacetic acid (EDTA). Acid flushing, on the other hand, affects soil fertility and causes changes in physicochemical composition due to mineral dissolution. Furthermore, because of its slow disintegration rate, the use of EDTA is hazardous to one's health and safety. This approach is further limited by the difficulty of retrieving heavy metal from the metal-EDTA complex. Surfactants could be used to help clean up soils that have been contaminated by metals and oils. Surfactants in solutions help to solubilize, disperse, and desorb pollutants while also allowing the soil to be reused (Hashim et al. 2011). In decontamination studies, a number of synthetic surfactants have been tested (Asci et al. 2008; Ellis et al. 1985; Nash et al. 1987). However, the necessity to replace synthetic surfactants with natural surfactants has led to biosurfactant research. Surfactin, rhamnolipids (both of bacterial origin), and sophorolipids (of yeast origin) have all been shown to have this capacity in research. Biosurfactants are good candidates for removing heavy metals from soil and sediment because of their ionic nature, biodegradability, low toxicity, and great surface characteristics. (Mulligan. 2009) suggests that varied concentrations of biosurfactants can be used to remove contaminants. According to (Das et al. 2009), cadmium removal using an aqueous solution happened at concentrations below the

CMC as well, while a concentration five times greater than the CMC resulted in the nearly full removal of 100 ppm of metallic ions. (Wen et al. 2009) investigated the degradation of a rhamnolipid in soils contaminated with cadmium and zinc and discovered that this substance might stay in the soil long enough to improve cadmium and zinc phytoextraction. Metal removal by ionic biosurfactants is considered to take place in the following order: 1) absorption of the biosurfactant to the soil surface and complexation with the metal; 2) detachment of the metal from the soil to the solution; and 3) interaction with biosurfactant micelles Electrostatic interactions trap heavy metals within micelles, which can be easily recovered using precipitation or membrane separation procedures (Kitamoto et al. 2002). Through ionic bonding, anionic biosurfactants form non-ionic compounds with metal. Because the metal-biosurfactant complex is desorbed from the soil matrix due to the reduction in interfacial tension, these connections are stronger than those between metal and soil. Cationic biosurfactants can compete for some, but not all, negatively charged surfaces, allowing them to replace similarly charged metal ions (ion exchange). Surfactant micelles can also remove metal ions from the soil surface.

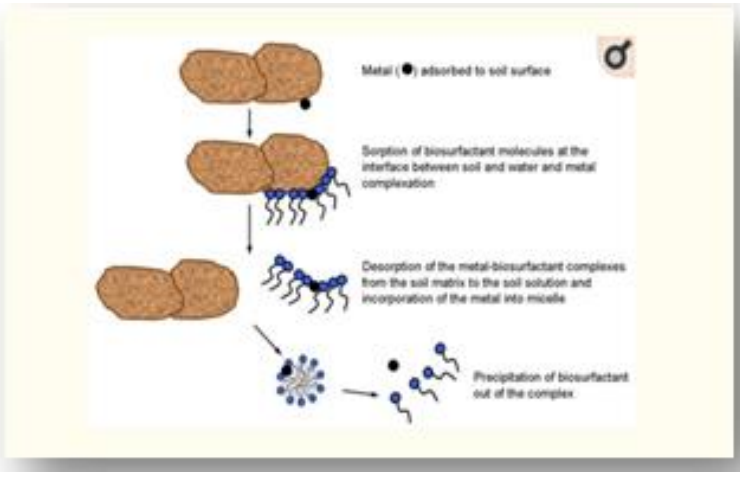


Figure 1.4. Biosurfactants in the Removal of Heavy Metals (Pacwa-Płociniczak. 2011)

1.2.5.5. Phytoremediation

Among the inorganic contaminants, heavy metals are the most problematic. They are harmful at high concentrations because they produce free radicals and cause oxidative stress. They also disrupted the normal function of some basic proteins by replacing them. In any way, the plant's potential for phytoremediation can be increased by using both metal-safe and biosurfactant-producing microscopic organisms. For example, the biosurfactant produced by *Bacillus* sp. J119 strain can improve the effectiveness of plant development in assault, sundagrass, tomato, and maize, as well as cadmium uptake. It is obvious from this analysis that the species selected for this purpose has a root colonization effect. For the remediation of excessive metals, an organism that aids the phytoremediation process is created (Hood and Zottola. 1995).

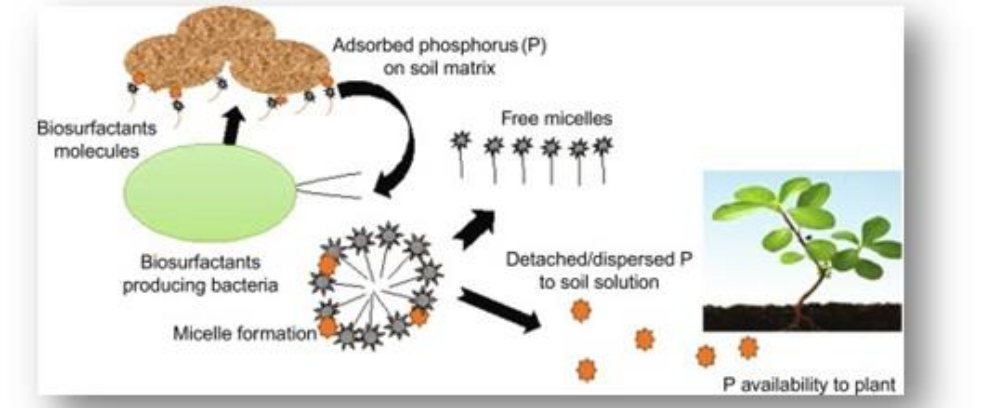


Figure 1.4. Biosurfactants in the Phytoremediation (Pacwa-Płociniczak. 2011)

1.2.5.6. Agriculture

Biosurfactants are also used in agriculture and play an important role in biocontrol components of microorganisms such as parasitism, antibiosis, rivalry, induced fundamental protection, and hypo-harmfulness. Surfactants can be used as preparation specialists to improve the dissolvability of bio-perilous chemical

combinations, such as PAH. The clear solvency of Hydrophobic Organic Contaminants is increased as a result of this (HOC). Surfactants are also reported to allow organisms to adsorb to toxins-affected soil particles, reducing the dispersion distance between the point of absorption and the site of biouptake by microbes. Surfactants are also used in horticulture for hydrophilization of heavy soils to achieve high wettability and even compost adsorption. They also prevent particular compost from hardening during capacity and speed up the distribution and entry of herbicide toxicants. The rhamnolipid biosurfactant, which is mostly produced by the *Pseudomonas* species, is known to have potent antibacterial properties. Furthermore, overall exposure to rhamnolipid biosurfactants is unlikely to have any negative effects on individuals or the environment. Fengycins have also been found to exhibit antifungal properties, suggesting that they could be used in the biocontrol of plant diseases.

1.2.5.7. Application of Biosurfactants in Cleaning of Oil Reservoirs

Heavy oils that settle at the bottom of storage tanks are highly viscous and cannot be removed with standard pumping so heavy oil residue requires solvent cleaning or even manual washing, both of which are risky, slow, and expensive processes. The use of biosurfactants in this cleaning procedure lowers the viscosity of the product and facilitates the creation of oil/water emulsions, making it easier to pump the residue and collect the crude oil once the emulsion breaks down (Singh et al. 2007; Mulligan and Wang. 2004). It is claimed that using biosurfactants instead of conventional surfactants in tank cleaning allows for the cleaning and recovery of 90% of the hydrocarbons in the residue (Mulligan. 2004). Rhamnolipid, a commonly isolated glycolipid biosurfactant, and surfactin, a lipoprotein type biosurfactant, are produced by *P. aeruginosa* and *B. subtilis*, respectively; these two biosurfactants have been shown by (Whang et al. 2008) to increase the solubility and bioavailability of a petrochemical combination and to stimulate

indigenous microorganisms for enhanced biodegradation of diesel-contaminated soil.

1.2.5.8. Antimicrobial Activity

Several biosurfactants have been shown to have antimicrobial activity in the literature for a variety of applications. Some biosurfactants are recognized to have medicinal potential as antibacterial, antifungal or antiviral substances. These characteristics make them suitable candidates for use as therapeutic agents and in the treatment of a variety of diseases. When compared to traditional antibiotics, biosurfactants' antimicrobial properties rely on alternative mechanisms to kill target organisms, and they predominantly kill bacteria by affecting the integrity of the plasma membrane or cell wall (Banat et al. 2010). The majority of antimicrobial glycolipid and lipopeptide-based biosurfactant compounds were obtained from microorganisms isolated from marine, terrestrial, and hydrocarbon-contaminated environments. Biosurfactant substances generated by bacterial strains recovered from wastewater are currently the subject of limited investigation. However, as compared to their synthetic equivalents, biosurfactants have a higher production cost, which limits their widespread application. The cost can be decreased by improving the strain, utilizing statistical methods to optimize the medium composition, or employing other, less expensive substrates. The selection of low-cost raw materials is critical to the process' overall economy, as they account for half of the final production cost and help lower waste treatment cost.

1.2.5.9. Anticancer Activity

Some microbial extracellular glycolipids cause cell separation rather than cell expansion in the human promyelocytic leukemia cell line; similarly, exposing PC 12 cells to MEL increased acetylcholine esterase movement and intruded on the cell cycle at the G1 stage, resulting in an abundance of neurites and incomplete cell separation; this suggests MEL causes neuronal separation and provide protection.

1.2.5.10. Antiviral Activity

The basic analogs of *C. bombicola*'s sophorolipid have been studied for its spermicidal, anti-HIV, and cytotoxic properties. The sophorolipid diacetate ethyl ester offshoot is the most potent spermicidal and virucidal specialist among the sophorolipids investigated.

1.2.5.11. Commercial Laundry Detergents

Surfactants, an important component of today's commercial garment cleaners, are all synthetically made and pose a threat to future aquatic life. The search for eco-friendly, characteristic alternatives for compound surfactants in garment cleaners has been re-energized by growing public awareness of the environmental problems and risks associated with compound surfactants. Biosurfactants such as Cyclic Lipopeptide (CLP) are stable across a wide pH range (7.0-12.0) and do not lose their surface-dynamic properties when heated to high temperatures. They demonstrated excellent emulsion creation capability with vegetable oils, as well as outstanding resemblance and strength to commercial clothes cleaners, indicating that they should be considered in a clothing cleansers strategy.

1.3. Importance and Aim of The Study

Pollution is widely acknowledged as one of the most pressing issues confronting human societies around the world, particularly in developing countries. Despite the fact that it is caused by man and his actions, it has negative consequences for human environments and resources (Al-Bager. 2005; Anandara and Thivakaran. 2010).

As a result, pollution and its consequences are regarded as one of man's greatest transgressions. Pollutants can cause primary damage by having a direct impact on the environment, or secondary damage by disrupting the delicate balance of the biological food chain in subtle ways that are only noticeable over time. The

most pressing concerns in today's society are the scarcity of raw materials, rising prices, and general pollution.

The challenges of recovering and recycling waste, as well as turning waste into valuable materials, are becoming more and more important. Environmental pollution, especially heavy metal pollution, has been defined as a serious environmental problem in recent years and numerous solutions have been discovered to remove it from the environment.

Surfactant use has increased 300 percent in the United States over the past 20 years. Surfactants are now produced over 3 million tons per year worldwide. Most of these surfactants can be chemically extracted from petroleum (Banat et al. 2000). Several bacterial and fungal species produce biosurfactants, a heterogeneous group of surfactants that are secreted intracellularly or extracellularly. These naturally occurring surfactants are amphiphilic, meaning they contain both hydrophilic and hydrophobic moieties, reduce surface and interface tension with high selectivity, low toxicity, and biodegradability. Emulsification, foaming, detergent and dispersibility are all facilitated by these properties (Sneha-Chakrabarti. 2013).

The production of biosurfactant by microorganisms has risen to prominence in recent years. Glycolipids, lipopeptides or lipoproteins, and polymeric components make up the majority of microbial surfactants. As a result, biosurfactants have received more attention in terms of identifying their potential applications. Surface activity, tolerance to pH, temperature, and ionic strength, biodegradability, low toxicity, emulsifying and demulsifying ability, and antimicrobial activity are all characteristics of microbial surfactants.

Isolation and identification of biosurfactant-producing bacteria from extreme soils, as well as characterization of the biosurfactant produced, were the aims of this study.

2. PREVIOUS STUDIES

In the study by (Ilori et al. 2005). *Aeromonas spp*. Biosurfactant manufacturing has been studied. When the emulsifying capabilities of the obtained biosurfactant were investigated, it was discovered that diesel (E24=65) was the best hydrocarbon substrate and hexane (E24=22) was the weakest. The medium containing glucose produced the highest amount of biosurfactant, whereas the media containing diesel and acetate produced the lowest amount, and soybean was found to be the best nitrogen source. At 5% NaCl content, pH 8.0, and 40oC, the biosurfactant's activity was at its peak.

It was stated in a study on the synthesis of biosurfactant from cassava waste water by *Bacillus subtilis* that biosurfactant production was performed at 30oC, 150 rpm shaking speed, airy circumstances, 4% inoculum density, and 75 mL of cassava waste water. According to (Nitschke and Pastore. 2006), the biosurfactant reduces the surface tension of the medium to 26.6 mN/m, the biosurfactant quantity is 3 g/L after 48 hours, and it maintains its surface-active qualities at 100oC, a high salt concentration of 20 g/L, and a wide pH range.

(Fernandes et al. 2007) investigated the antimicrobial efficacy of the biosurfactant generated by *Bacillus subtilis* R14 strain against bacteria with multiple antibiotic resistance. The surface tension of the medium was found to be 54 mN/m at the start of microbial growth on specified media and dropped to 30 mN/m after 20 hours. After 40 hours of growth, a crude surfactant concentration of 2.0 g/L was attained. It has been proposed to make two surfactants for preliminary characterization. Surfactants' antimicrobial activity was tested against 29 microorganisms to determine their antimicrobial activity. A well-defined drug resistance profile was observed in *Enterococcus faecalis* (11 strains), *S.aureus* (6 strains), *Pseudomonas aeruginosa* (7 strains), and *E.coli* CI 18 (1 strain). Surfactants were found to be toxic to all strains, especially *Enterococcus faecalis*.

When alternative carbon and nitrogen sources were tested in the manufacture of biosurfactants by *Pseudomonas fluorescens*, the best results (C:N ratio 10) were obtained using olive oil as the carbon source and ammonium nitrate as the nitrogen source. In 36-48 hours, the surface tension dropped below 32 dyne/cm, and the emulsification index (E24) was calculated to be 65 %. Separation by acetone precipitation was used to study the characteristics of the biosurfactant. The biosurfactant produced a positive rhamnosis test result, as does the rhamnolipid type in nature. The biosurfactant has been shown to have good foaming, emulsifying, and antimicrobial characteristics. It remained stable when exposed to high temperatures (15 minutes over 120^{o}C), high salinity (10% NaCl), and a wide pH range. Simultaneous infrared spectroscopy (FTIR) and HPLC analyses were carried out (Abouseoud et al. 2008).

In a study on biosurfactant synthesis by *Bacillus subtilis* 20 B, (Joshi et al. 2008a) used 7 g/L $(NH_4)2SO_4$, 3.8 g/L $Na_2HPO_4.12H_2O$, 0.7 g/L $MgSO_4.7H_2O$, and 0.5 g yeast extract. Furthermore, 2% (w / v) glucose, maltose, lactose, and sucrose were added as carbon sources, as was 2% heptane from hydrocarbons, kerosene, paraffin oil, cottonseed oil, and alcohols methanol, ethanol, and propanol. The studies were conducted in a 5 L fermenter with 3 L of medium, at 30^{o}C, under air conditions, with a stirring speed of 300 rpm, in the pH range of 6.8-7.2, and at a 2% inoculum level. It has been discovered that the best medium for biosurfactant production contains 2% glucose, and the fermentation period is 8-10 hours. The surface tension of the biosurfactant is lowered to 29.5 mN/m, and it is stable throughout a wide pH, temperature, and salt concentration range.

(Joshi et al. 2008b) studied biosurfactant production conditions from *Bacillus licheniformis K51, Bacillus subtilis 20B, Bacillus subtilis R1* and *Bacillus spp., HS3* bacteria using molasses and whey. Maximum biosurfactant generation has been reported in 5-7 % (w/v) molasses containing medium, pH 7, 45^{o}C, 2% inoculum density, and 160 rpm shaking speed. It has been found that

after 9 days of incubation at 80°C and a wide pH and salt concentration range, the biosurfactant retains its surface-active capabilities.

Bacillus subtilis MUV4 synthesized biosurfactant in a 200 rpm shaking incubator at 30°C in modified Mckeen medium containing 1% glucose, 1% monosodium glutamate, and 0.3 % yeast extract. After 48 h of incubation, *Bacillus subtilis* MUV4 supernatant reduced the surface tension of the medium from 53.50 mN/m to 33.50 mN/m. The crude biosurfactant was dissolved in methanol and vacuum dried. The activity of the remaining biosurfactant in the supernatant, crude biosurfactant, and crude methanol was in the pH ranges of 1-6, 7-10, and 4-10, respectively, and the emulsion stability (E24) at pH 7.0 was 66.67%, 33.333%, and 33.333%, respectively. After 5 h of incubation at +4°C, room temperature, and 50°C, the supernatant and crude biosurfactant demonstrated surface tension activity. The leftover biosurfactant in crude methanol, on the other hand, demonstrated surface tension activity however after 5 h at 100°C. The surface tension activity of the supernatant and crude biosurfactant was stable at 3-10% NaCl, while the remaining biosurfactant in crude methanol was stable at 3-20% NaCl. However, at NaCl concentrations more than 5%, all samples lost emulsion stability (Suwansukho et al. 2008).

(Das et al. 2009) found that the carbon source affected biosurfactant production qualitatively and quantitatively in their study on the ability of *Bacillus circulans* to generate biosurfactants from diverse carbon sources. The highest biosurfactant production by carbon source take placed in the following order: glycerol, starch, glucose, and sucrose in that order.

The biosurfactant producing bacteria was obtained from the oil field and recognized as *Bacillus subtilis* HOB2 using 16S rRNA gene sequencing. *Bacillus subtilis* HOB2 biosurfactant synthesis was examined utilizing various carbon and nitrogen sources under thermophilic and mesophilic conditions. After 24 hours of incubation, the strain developed and produced surfactant by lowering the surface tension of the glucose medium to 28 mN/m and the surface tension of the sucrose

medium to 27 mN/m. When ammonium ions were applied as the nitrogen source, maximum biosurfactant synthesis was achieved. When exposed to 100°C, the surfactant remained stable at high salinity (25 percent NaCl) and throughout a wide pH range (pH 5.0-11.0). The biosurfactant's emulsification index (E24) with kerosene was calculated to be 68 %. Kinetic researchers have indicated that biosurfactant synthesis is a cell growth-related activity. First and foremost, chemical characterization, TLC, and IR analyses indicated that surfactant had a lipopeptide composition identical to surfactant. *Bacillus subtilis* HOB_2 biosurfactant qualities are expected to be exploited in commercial applications such as oil recovery, bioremediation of marine and soil environments, and the food sector (Haddad et al. 2009)

(Nasr et al. 2009) evaluated the characteristics of the new biosurfactant generated by *Bacillus* spp. strains in their investigation. It was discovered that 16 of the 102 isolates tested positive for hemolytic activity, 32 tested positive for oil spreading, and 16 tested positive for oil precipitation. In total, 14 isolates, or 13.72 % of those that showed favorable qualities in more than one of the procedures listed above, were approved as biosurfactant producers. The surface tension of 14 isolates varied in the range of 23.3-57.6 mN/m, and the activity of emulsion (E24) in diverse hydrocarbon sources (kerosene, n-hexadecane, petroleum) varied in the range of 0-100 0%, according to the results of these investigations. In accordance with the findings, biochemical characterizations of two-gram positive isolates (SN1 and SN12), widely regarded as the top biosurfactant producers, were performed using a variety of tests. These two strains with 16S rDNA sequence analysis were *Bacillus spp.* Among the species, SN1 was defined as *Bacillus subtilis* and SN12 as *Bacillus cereus*.

Oil-contaminated soil was gathered from four separate Indian car shops. *Bacillus subtilis* and *Pseudomonas aeruginosa* were isolated from this soil samples, and their biosurfactant production was tested using the oil smear technique and the emulsification stability test with vegetable oil, kerosene,

petroleum, and gasoline. The best biosurfactant production of *Bacillus subtilis* and *Pseudomonas aeruginosa* was achieved using vegetable oil, kerosene, petroleum, and gasoline as sources. TLC was used to identify the separated biosurfactants. The optimal source for biosurfactant production of *Bacillus subtilis* and *Pseudomonas aeruginosa* was revealed to be diesel, and the biosurfactant activity of *Pseudomonas aeruginosa* was better than that of *Bacillus subtilis* (Priya and Usharani. 2009).

The production of bioemulsifier by *Bacillus licheniformis* PTCC 1595 was studied. *B. licheniformis* was cultivated in nutrient broth, and bioemulsifier production was measured using surface tension and the emulsification index every 24 hours (E24). Then, in addition to the nutrient broth medium, *Bacillus licheniformis* PTCC 1595 was grown under multiple conditions to maximize bioemulsifier synthesis. Nutrient broth medium including starch, Fe+2, Mn+2, and olive oil was proven to be the optimum culture media. Following growth, the microbial mass was extracted from the supernatant using the acid precipitation method (Noudeh et al. 2010).

(Chander et al. 2012) used the *Bacillus subtilis* MTCC 441 strain to produce biosurfactant, and its biosurfactant efficacy was tested against several vegetable edible oils. The parameters were improved and the manufacture of the biosurfactant was carried out, in order to improve its growth. When grown at mesophilic temperatures, pH 7 and in sesame oil it had the highest biosurfactant activity. With sesame oil, the biosurfactant demonstrated a high emulsification index of 71%.

Twelve different solid substrates were evaluated for the synthesis of *Bacillus subtilis SPB1* biosurfactant, with millet yielding the highest yield. The Plackett-Burman design was used to assess the impact of five factors (temperature, humidity, initial pH, inoculum age, and inoculum amount). Temperature, inoculum age, and moisture content all had a positive effect on SPB1 biosurfactant synthesis, according to statistical analyses. The optimal moisture content, inoculum age, and

temperature were 88 %, 14 hours, and 37^{o}C, respectively. The biosurfactant's antimicrobial activity was evaluated by testing it against 11 bacteria and 8 fungi. The findings revealed that the biosurfactant had high antimicrobial effects against multi-drug resistant pathogens (*S.aureus, Staphylococcus xylosus, Enterococcus faecalis*, and *Klebsiella pneumonia*) (Ghribi et al. 2012).

The biosurfactant formed by the *Bacillus subtilis* strain isolated from crude oil samples was categorized and its features were compared with commercially available chemical surfactants in the research by (Vaz et al. 2012). surface tension (29.0 mN/m) and critical micelle concentration (40 mg/l) were found to be similar to previously reported values for surfactin. The biosurfactant's temperature and pH stability were also assessed. After two weeks of exposure to different temperatures (20, 37, 46oC), the biosurfactant was found to be as stable as commercial surfactants Glucopone® 215, Glucopone® 650, Findet® 1214N/23, and linear alkylbenzene sulfonates (LAS). Furthermore, no significant loss in surface activity of the biosurfactant exposed to 121oC for 20 minutes was reported. Commercial chemical surfactants were stable across a broad pH range (pH 3.0-10.0), whereas biosurfactants were unstable below pH 5.0. When compared to commercial chemical surfactants, the emulsification index revealed that the biosurfactant had the same or greater capacity to emulsify with n hexadecane. The biosurfactant was found to be antimicrobial toward *S.aureus* and *E.coli*. According to the findings of this study, *Bacillus subtilis* EG1 biosurfactant could be used as an alternative to chemical surfactants in a variety of industries.

(Pathak and Keharia. 2013) discovered that the *Bacillus subtilis* K1 strain isolated from the aerial roots of the banyan tree produces a highly heterogeneous mixture of surfactin, iturin, and fengisin. The extracellular extract, which was made up of cyclic lipopeptides, demonstrated very good emulsification activity as well as excellent emulsion stability. After 48 hours of incubation in Luria Broth medium, the biosurfactant activity was at its peak. The emulsion of hexane, heptane, and octane prepared from the 48-hour culture remained stable for 2 days, while the

emulsion of 4-stroke engine oil remained stable for more than a year. Crude lipopeptide biosurfactant extracted by acid precipitation had a critical micelle concentration of 20.5 g/mL. The biosurfactant activity was stable for 2 hours at 100oC, 6-12 hours in a wide pH range, and at NaCl concentrations greater than 10% (w/v). The use of biosurfactant in a sand pack column saturated with 4-stroke engine oil at the laboratory scale increased oil recovery by approximately 43 %, demonstrating the biosurfactant's suitability for microbially enhanced oil recovery.

(Pereira et al.2013) optimized biosurfactant production of three strains of *Bacillus subtilis* (309, 311, and 573) isolated from Brazilian crude oil in terms of different carbon and nitrogen sources. In three strains, the lowest surface tension values were obtained using sucrose-containing media. Fourier Transform Infrared Spectroscopy (FTIR), Proton Nuclear Magnetic Resonance (1H NMR), and Matrix Assisted Laser Ionization Mass Spectroscopy strain (MALDI-TOF) were used to characterize the biosurfactant produced by each. Chemical analysis revealed that these three isolates produced very similar mixtures with varying ratios of C13-C14-C15 surfactant. The structure of the surfactant produced by the three strains has been shown to be compatible with their surface activities. The interfacial activities of biosurfactants were compared to those of chemical surfactants. It was also compared in terms of oil recovery, and it was determined that biosurfactants had better surface activities and oil recovery effects than chemical surfactants.

The goal of the study was to characterize and optimize the growth media for *Serratia rubidaea* SNAU02, which was isolated from hydrocarbon-contaminated soil in Cuddalore district, Tamilnadu, India in 2013 by nalini and parthasarathi. The biosurfactant produced by *S. rubidaea* SNAU02 reduced the surface tension in MSM medium to 34.4 mN m1. The biosurfactant was characterized using FT-IR and GC–MS. Dirhamnolipid was found to be a more abundant congener than monorhamnolipid in the GC–MS analysis. The response surface methodology (RSM) -central composite design (CCD) was used to optimize the media for biosurfactant production. The emulsification index was

highest when 29.31 g L1 mannitol, 2.06 g L1 yeast extract, medium pH 6.97, and 5.69 g L1 NaCl were used. The biosurfactant from *S. rubidaea* recovered 92 percent of used engine oil that had been adsorbed to a sand sample, implying that it could be used in microbial enhanced oil recovery and bioremediation.

Biosurfactants are excellent substitutes for chemical surfactants because they are non-toxic, biodegradable, and cost effective. The biosurfactant production from two isolates, *Bacillus amyloliquefaciens SAS-1* and *Bacillus subtilis* BR-15, was studied by (Sharma et al. 2018). Fourier transform infrared spectroscopy and mass spectrometry identified the biosurfactants as lipopeptides and surfactins with molecular weights of 1007, 1021, 1035, and 1049 Da. These molecules emulsified hydrocarbons well (E24 60–78%) and were stable at a wide range of temperatures (4 –100°C) and pH (4 –10). Because of their high surface activity, the biosurfactants produced by SAS-1 and BR-15 increased oil recovery by 56.91 ± 1.52 and $66.31 \pm 2.32\%$ in a sand pack column experiment, respectively. Furthermore, biosurfactants effectively increased engine oil degradation with microbial consortium by more than threefold (75–94%) when compared to that without biosurfactants (22–31%). As a result, the strains presented have a lot of potential in terms of environmental applications like improving oil recovery and bioremediation of oil spills.

Bacillus cereus was grown in a mineral medium containing 2% frying oil and 0.12% peptone to produce a biosurfactant in this study by (Durval et al. 2020). Surface tension was achieved at 28.7, 27.5, and 32 mN/m, and biosurfactant concentrations were 4.3, 4.6, and 4.7 g/L, respectively, when the production was scaled up from flasks to 1.2-, 3.0-, and 50-L bioreactors. The biosurfactant was identified as anionic, and its lipopeptide nature was discovered through nuclear magnetic resonance, thin layer chromatography, and gas chromatography analyses. Toxicity tests revealed that the fish *Poecilia vivipara* and the bivalve *Anomalocardia brasiliana* had survival rates of more than 90% and 55%, respectively, indicating that this biosurfactant could be used to clean up the marine

environment. Furthermore, in bioassays performed in seawater, the biosurfactant stimulated the growth of autochthonous microorganisms regardless of the presence of motor oil. These findings show that the biosurfactant is biocompatible and has the potential for industrial-scale production and use in bioremediation of polluted marine environments caused by oil spills.

(Samsu et al. 2020) conducted research to extract and characterize biosurfactants produced by strains isolated from used engine oil-contaminated soil. Biosurfactants from three isolates (2c, 2k, and 2m) were extracted to see how stable they were over a range of pH and temperature. The antimicrobial activity of biosurfactants against selected Gram-positive and Gram-negative bacteria was investigated using Fourier transform infrared spectroscopy to determine the functional group of the extract. After being exposed to temperatures ranging from 27 °C to 70 °C and pH levels ranging from 4 to 12, the biosurfactants demonstrated good stability in an oil displacement assay. The antimicrobial activity of the extracts of isolates 2k and 2m was also discovered through *Bacillus subtilis* inhibition. Biosurfactants from isolates 2c and 2m were identified as possibly belonging to the rhamnolipid group, with O–H functional groups ranging from 3550 to 3200 cm1. Meanwhile, isolate 2k's biosurfactants were likely lipoptide biosurfactants, as the N–H functional group was observed at 3400 to 3300 cm1. As a result, the three isolates chosen were found to be potential producers of rhamnolipid and lipopeptide biosurfactants, and the extract was found to be stable across the pH and temperature ranges tested.

Antiseptics and disinfectants are frequently used to kill microorganisms. Biofilm-dwelling microorganisms, on the other hand, are less susceptible to biocide treatment and, in some cases, resistant to it. Singh and Sharma's study from 2020 describes the isolation and characterization of a lipopeptide biosurfactant with disinfectant-like activity. *Bacillus tequilensis* strain SDS21, an endo-rhizospheric bacterium, produced biosurfactant. With a CMC of 40 mg/l, biosurfactant reduced the surface tension of water from 72 to 30 mN/m. Biosurfactant was found to be a

mixture of C14, C15, C16, and C17 surfactin homologues after Liquid Chromatography–Mass Spectrometry analysis. The lipopeptide biosurfactant had bactericidal activity against planktonic cells and sessile cells that were part of a biofilm. On polystyrene, glass, and stainless-steel surfaces, the biosurfactant treatment removed more than 99 percent of bacterial biofilm. Even after being exposed to extreme conditions such as high temperature and extreme pH, the biosurfactant retained its bactericidal and biofilm eradicating properties. Biosurfactant retained its bactericidal and biofilm-removing activity even in hard water containing Mg^{2+} and Ca^{2+} ions, unlike some commonly used disinfectants. As a result, the biosurfactant produced by SDS21 can be used as a disinfectant or in disinfectant-like formulations that are effective against planktonic and biofilm-residing bacteria.

Bacillus oceanisediminis H2, isolated from tannery processing industrial wastelands, was successful in producing a crude biosurfactant when grown on biosurfactant production media supplemented with glucose as a carbon source and urea as a nitrogen source, according to a study published in 2021 by Banerjee and Ghosh. The crude biosurfactant was purified using a combination of silica-gel column chromatography and thin layer chromatography (TLC) before being lyophilized for further testing. The Rf values of the crude and purified biosurfactant were 0.678 (of one spot) and 0.614, respectively, according to TLC analysis. FTIR and LC-MS analysis of the solid purified product confirmed that the purified biosurfactant is surfactin by nature. Surface tension of the crude biosurfactant produced is 46.909 mN/m, compared to 72 mN/m for water.

Heavy metal toxicity has emerged as a serious ecological issue that has a negative impact on ecosystems due to bioaccumulation, posing a serious public health risk. To decontaminate and restore metal-contaminated areas, many traditional strategies have been developed and implemented. However, because of their high operational costs, high energy requirements, post-waste disposal issues, and secondary pollutant generation, these traditional approaches are not very

acceptable and environmentally safe for heavy metal remediation. Because of its biodegradation capability, economic effectiveness, and ecofriendly nature, biosurfactant-based heavy metal bioremediation is a sustainable and promising approach. (Mishra et al. 2021) isolated *Pseudomonas* sp., *Bacillus* sp., *Citrobacter freundii,* and *Candida tropicalis* as potential biosurfactant sources that produce surfactin, rhamnolipids, and sophorolipids. Because of the severity of heavy metal pollution in some areas of the environment, biosurfactants have attracted a lot of interest and attention as a new century's emerging multi-functional technology for removing heavy metal pollutants. The role of biosurfactants in the bioremediation of heavy metals from contaminated environments is discussed in this study. Furthermore, the biosurfactant–metal complexation and metal remediation interaction mechanisms are discussed. Based on the literature review, more research is needed to elucidate the mechanistic roles of biosurfactants and to investigate structural characterization and gene regulation in order to increase their productivity and bioremediation applicability.

Toxicity of agricultural soil as a result of petroleum contamination has recently become a major concern. The presence of various hazardous hydrocarbons in petrol oil causes toxic effects in agricultural crops. Researchers have studied the degradation of petroleum hydrocarbons extensively, indicating the need for effective treatments for the detoxification of petroleum contaminated soil and its reuse for crop production. Thus, secondary metabolites "biosurfactant" (natural surfactant) and the potent plant growth promoting (PGP) bacterial strain *Pseudomonas* sp. SA3 were used in the designed treatments for growing agricultural crop by (ambust et al. 2021. The strain's biosurfactant has a 43% emulsification capacity and a 34.5 mN/m surface tension reduction ability, while the plant growth promoting traits show a 93.46 g/mL phosphate solubilization ability, 69.41% siderophores (iron chelating compound) production ability, and 81.41 g/mL indole acetic acid (IAA) production ability. Furthermore, the results of the design treatments indicate that treatments amended with the strain

SA3 and biosurfactant are effective in the management of petroleum contaminated soil, indicating treatment EX 5 (1 kg soil + 1 L water + *Pseudomonas sp*. SA3 + 300 mL crude biosurfactant) as an efficient treatment in increasing phytochemical constituents and improving growth parameters by 10–15 %. As a result, the developed treatments can be used to effectively manage petroleum contaminated soil for agronomy.

(Prakash et al. 2021) conducted a study to evaluate the coupling of bioremediation (BIO) and electrokinetic (EK) remediation of crude oil hydrocarbons using the bio-electrokinetic (BIO-EK) technique. By increasing the solubility of organic materials, the use of bacterial biosurfactant (BS) may improve remediation efficiency. *Bacillus subtilis* AS2, *Bacillus licheniformis AS3*, and *B.velezensis* AS4 were isolated and identified as potential biosurfactant producing marine bacteria in this study using 16S rDNA analysis. With the optimum temperature of 37 °C and pH 7, the biodegradation efficiency of crude oil was found to be 88 %, 92 %, and 97 % for strains AS2, AS3, and AS4, respectively. In nature, FTIR confirms that BS is a lipopeptide. According to GCMS, three isolates effectively degraded the crude oil from lower to higher molecular weight (C8 to C28). The use of BS in electokinetic remediation increased the biodegradation rate of crude oil contaminated soil by (92 %) in two days compared to EK (60 %). The presence of higher total organic content in the BS than in the EK confirms that BS improves hydrocarbon solubilization and leads to faster electromigration of hydrocarbon to the anodic compartment. This study demonstrated that combining BIO-EK with BS can improve in situ bioremediation of petroleum-contaminated soils

3. MATERIAL AND METHODS

3.1. Materials

3.1.1. Microorganisms

The types of bacteria used in this study were isolated from samples collected from different regions in the Arab Republic of Egypt, from north to south, starting from the city of Dahab, which is a tourist city in the South Sinai Governorate, located on the Gulf of Aqaba, southeast of the Sinai Peninsula, about 100 km away from the city of Sharm Sheikh and 87 km from the city of Nuweiba, and it was named after the color of its golden sand. The city is famous of its pristine beaches and natural diving sites rich in coral reefs. after that the Red Sea Governorate, in which samples were collected from 3 regions, the first of which is the city of Ras Ghareb, located on the western bank of the Gulf of Suez and 150 km north of Hurghada. Its economy, in addition to tourism, depends on the extraction of oil. There are several offshore platforms next to it in the extended Gulf of Suez that flow into its storage. Then the Umm Ghaig area, which is located south of the city of Qusair on the Red Sea coast and contains 105 million tons of reserves of minerals, the most important of which are zinc and lead. The last area in the Red Sea Governorate is the Hamraween port area, which is located on the Red Sea coast, about 20 km north of Quseir and 60 km south of Safaga. The port's activity is focused on the export and shipment of mineral ores (phosphates - amines). Then some samples were collected from several cities on the banks of the Nile from north to south, starting with the city of Samanoud, which is located in the north-east of Gharbia Governorate and is characterized by an excellent geographical location on the banks of the Damietta branch of the Nile. Then the city of Qeft, which is located in the Qena Governorate, about 20 km south of the city of Qena, and about 43 km north of the ancient city of Luxor, on the eastern side of the Nile River. Next is the Aswan Governorate, in which samples were collected from 4areas, the first of which is the city of Edfu, which is located in the north of Aswan Governorate and is about 100 km from the city of Aswan and is

located west of the Nile River and has many archaeological areas that belong to the ancient Egyptian state and the Ptolemaic era. Then the industrial zone in the city of Aswan, which is considered the fortress of craft workshops and factories in the east of the city, where Aswan Governorate is considered the land of giant projects in Upper Egypt and the capital of the African economy, as it possesses a huge stock of various natural raw materials such as granite, phosphate, marble, manganese and gold. Then the area of the High Dam spillway, which is located behind the High Dam, 15 km south of Aswan, which is a water source used to control the water level in the dam and allows water to drain before it exceeds the permissible design limit. The soil in this area is characterized by a high degree of salinity. Finally, the tourist village area south of Aswan, which is characterized by its soil with a very high degree of salinity.

3.1.2. Types of Media Used

3.1.2.1. Nutrient Broth and Plate Count Agar

Plate count Agar was used for the isolation of *Bacillus* species and for the storage of stock cultures, and Nutrient Broth medium was used to activate the isolates (Tables 3.1 and 3.2) (Nadeem et al. 2007; Joshi. 2010).

Table 3.1. Composition of Nutrient Broth medium

Medium composition	g/L
peptone	5.0
Beef extract	3.0

Table 3.2. Composition of plate count Agar medium

Medium composition	g/L
Peptone from casein	5.0
Yeast extract	2.5
D (+) glucose	1.0
Agar	14.0

After the components of the medium were dissolved in distilled water (pH=7.0±0.2), they were sterilized at 121°C at 1.2 atm pressure for 15 minutes.

3.1.2.2. Gsp Agar (*Pseudomonas Aeromonas* Selective Agar Base)

Medium proposed by KIELWEIN (1969 - 1971) for detecting *Pseudomonas , Aeromonas , Enterobacteriaceae* and others in foodstuffs as well as in wastewater and equipment of the food industry.

Table 3.3. Composition of GSP Agar

Medium composition	g/L
Sodium L(+)glutamate	10.0
Soluble starch	20.0
Potassium dihydrogen phosphate	2.0
Magnesium sulfate	0.5
Phenol red	0.36
Agar	12.0

After the components of the medium were dissolved in distilled water (pH=7.0±0.2), they were sterilized at 121°C at 1.2 atm pressure for 15 minutes. cool to 45-50°C. Add 100,000 IU sodium penicillin g/L and, if required, 0.01 g pimaricin/L.

3.1.2.3. Blood Agar

Blood agar is used for the isolation and cultivation of various fastidious microorganisms and to determine the haemolytic activities of biosurfactant producing bacteria (Table 3.4) (Karthik et al. 2010; Noudeh et al. 2010).

Table 3.4. Composition of Blood Agar

Medium composition	g/L
Nutrient substrate (heart extract and peptones)	20.0
Sodium chloride	5.0
Agar	15.0
Blood	50-80 mL

After the components of the medium were dissolved in distilled water (pH= 6.8 ± 0.2), they were sterilized at 121°C at 1.2 atm pressure for 15 minutes. cool to 45-50 °C. add 5-8 % defibrinated blood.

3.1.2.4. Mueller Hinton Broth and Mueller Hinton Agar

In order to determine the antimicrobial (antibacterial) activity of the biosurfactant producing bacteria, Mueller -Hinton Broth, Mueller -Hinton Agar were used to revive the isolates taken from the stock culture (pathogenic bacteria), while disc diffusion method was used to determine the antimicrobial activity (Table 3.5, Table 3.6) (Fernandes et al. 2007).

Table 3.5. Composition of Mueller Hinton Broth

Medium composition	g/L
Meat infusion	2.0
Casein hydrolysate	17.5
Starch	1.5

Table 3.6. Composition of Mueller Hinton Agar

Medium composition	g/L
Meat infusion	2.0
Casein hydrolysate	17.5
Starch	1.5
Agar	13.0

3.1.2.5. Malt Extract and Potato Dextrose Agar

To determine the antimicrobial (antifungal) activity of the biosurfactant producing bacteria, malt extract and Potato Dextrose Agar were used to revive the isolates taken from the stock culture (pathogenic fungi), while disc diffusion method was used to determine the antimicrobial activity. (Table 3.7) (Fernandes et al. 2007). For the malt extract broth, we use 17 g/L.

Table 3.7. Composition of Potato Dextrose Agar

Medium composition	g/L
Potato infusion	4.0
D (+) Glucose	20.0
Agar	15.0

3.1.2.6. Biosurfactant Production Culture Medium

A mineral salt medium with the following content (g/L) was used for biosurfactant production: 2.5 g/L $NaNO_3$, 0.1 g/L KCl, 3.0 g/L KH_2PO_4, 7.0 g/L K_2HPO_4, 0.01 g/L $CaCl_2$, 0.5 g/L $MgSO_4.7H_2O$) supplemented with 5 mL trace element solution (0.116 g/L of $FeSO_4.7H_2O$, 0.232 g/L of H_3BO_3, 0.41 g/L of CoCl2.6H2O, 0.008 g/L of $CuSO_4.5H_2O$, 0.008 g/ L of $MnSO_4.H_2O$, 0.022 g/L of [NH4]6Mo_7O_{24}, 0.174 g/L of $ZnSO_4$) then supplemented with 5% olive oil as substrate and autoclaved for 15 minutes at 121°C. The medium was then inoculated with 5% of the bacterial broth and cultured for 3 days at 37°C and 150 rpm (El-Sheshtawy. 2016).

3.1.3. Chemicals and Solutions Used in Biosurfactant Analysis

3.1.3.1. Crude Oil

Obtained from the Department of Automotive Engineering Cukurova University. And used in the Oil spread technique analysis of the produced biosurfactant.

3.1.3.2. HCl and NaOH Solution

For the extraction of biosurfactant produced in liquid medium and optimization of Biosurfactant produced by the isolated bacteria, 6 M HCl and 0.01 M NaOH were prepared.

3.1.3.3. Chloroform and Methanol

For extraction of biosurfactant produced in broth we used chloroform: methanol (2:1).

3.2. Methods

3.2.1. Isolation of Bacterial Strains from Soil Samples

3.2.1.1. Isolation of *Bacillus* Species

For the isolation of biosurfactant-producing *Bacillus* species from soil samples were taken from different regions of Egypt, 1 g soil was added to 10 mL sterile distilled water, mixed homogeneously, and then heated at 85ºC for 10 minutes. temperature was applied in a water bath. After temperature treatment, cultivation was made on plate count agar media to obtain a single colony and incubated at 37 ºC for 1 night. After incubation, single dropped colonies in the medium were evaluated according to their morphology, and colonies with different morphologies were cultivated on plate count agar media by streaking and incubated at 37 ºC.

3.2.1.2. Isolation of *Pseudomonas*, *Aeromonas, Enterobacteriaceae* and Others

For the isolation of biosurfactant-producing *Pseudomonas, Aeromonas, Enterobacteriaceae* and other species from soil samples were taken from different regions of Egypt, 1 g soil was added to 10 mL sterile saline (0.9% NaCl) water, mixed homogeneously, and then cultivation was made on GSP Agar media contains (100,000 IU sodium penicillin g/L) to obtain a single colony and incubated at 37 ºC for 1-5 night. After incubation, single dropped colonies in the medium were evaluated according to their morphology, and colonies with different morphologies were cultivated on GSP Agar media contains (100,000 IU sodium penicillin g/L) by streaking and incubated at 37ºC.

3.2.2. Biosurfactant Production in Liquid Media

For biosurfactant production, the bacterial sample in nutrient agar medium was inoculated from the solid medium into 10 mL Nutrient broth for resuscitation and incubated in a shaking incubator at 150 rpm for 1 night at 37ºC.After the incubation, the bacterial culture was inoculated at a rate of 10% in 100 mL medium (Biosurfactant production Culture medium), pH 7.0 containing 5% olive oil and incubated in a shaking incubator at 150 rpm for 72 hours at 37ºC. At the end of the incubation, bacterial cells were separated from the culture broth by centrifugation (Sigma 2-16 K centrifuge) at 9000 rpm for 20 minutes (pellet with supernatant). The supernatant was stored at +4ºC for use in biosurfactant analysis (Fernandes et al. 2007).

3.2.3. Screening of Biosurfactant Produced By The Isolates

The biosurfactant activity of the isolates was determined using complementary and confirmatory procedures such as the haemolysis test, drop collapse, oil spreading, surface tension (ST) test, and emulsification activity.

3.2.3.1. Haemolytic Activity

3.2.3.1.(1). Haemolytic Activity of The Biosurfactant-Producing Bacteria

Fresh colonies were taken and smeared on blood agar plates containing 7 % v/v human blood. These plates were incubated at 37°C for 1-7 days The plates were then observed for the presence of clear zone around the colonies which indicating that biosurfactant-producing organisms were present. Alpha haemolysis or partial haemolysis is indicated by the appearance of a partial transparent zones beneath the colonies. Beta or complete haemolysis of the blood cells is indicated by a yellow transparent zone around the colony. Gamma or no haemolysis is shown by no change in the blood agar plates. For biosurfactant synthesis, alpha and beta haemolysis were regarded positive (Alvarez. 2015).

3.2.3.1.(2). Haemolytic Activity of The Crude Biosurfactant Solution

Blood agar media containing 7 % v/v human blood was inoculated with pure biosurfactant solution, sterile empty antibiotic discs were placed in the Blood agar media after absorbing the pure biosurfactant solution at 50 µL concentration and incubated at 37°C for 24 - 48 hours. At the end of incubation, the plates were then observed for the presence of clear zone around the discs. Alpha haemolysis (partial) is indicated by the appearance of a partial transparent zones beneath the colonies. Beta haemolysis (complete) of the blood cells is indicated by a yellow transparent zone around the colony. Gamma or no haemolysis is shown by no change in the blood agar plates. The zone diameter around the discs was measured.

3.2.3.2. Surface Tension

The surface tension of the cell free culture broth obtained after 24 h, 48 h, 72 h and 15 days of incubation was measured by using the Optical Contact Angle / Surface Tension Measurement Device (TDIC - LAUDA) and the Ring method (Accorsini et al. 2012) in Cukurova University, Faculty of Science and Letters, Biology Department, Bacteriology Laboratory.

3.2.3.3. Oil Spread Technique

The technique described by (Morikawa et al. 2000) was used to perform the oil spreading experiment. In brief, 30 mL of distilled water was added to a petri dish of 90 mm diameter followed by addition of 100 µL of crude oil to the surface of the water. 20 µL of cell free culture broth was then added to the oil surface. If biosurfactant is present in the cell-free culture broth, the oil will be displaced by an oil-free clearing zone, the diameter of which shows the surfactant activity, also known as oil displacement activity. The negative control was distilled water (without surfactant), in which no oil displacement or clean zone was seen, while the positive control was tween 80.

3.2.3.4. Emulsification Activity

Emulsification capacity of the biosurfactant towards Hydrocarbons such as xylene, n-heptane and sunflower oil was done using the (Cooper and Goldenberg method. 1981). A mixture of 2 mL hydrocarbon and 2 mL cell free extract obtained after the centrifugation of sample culture were taken in a test tube and homogenized by vortexing at high speed for 2 min. The emulsion activity was investigated after 1 hour, 24 hours and 1 week and the emulsification index (E24) was calculated using following formula: E24 (%) = total height of the emulsified layer/total height of the liquid layer × 100.The results were compared with tween 80 as positive control and distilled water as a negative control.

$$E24(\%) = \frac{Total\ height\ of\ the\ emulsified\ layer}{Total\ height\ of\ the\ liquid\ layer} x\ 100$$

3.2.3.5. Drop Collapse Assay

On a clean glass slide or a clean glass petri dish, a single droplet of oil and supernatant was placed. After 1 minute, the shape of the drop on the oil surface was examined. The presence of surfactant in the culture supernatant causes the

drop to collapse, and if the drops remain intact, the result is negative. Tween 80 was utilized as a positive control and distilled water as a negative control (Korayem. 2015). The diameter of the oil drop is measured before and after adding the supernatant. The presence of surfactant reduces the interfacial tension between the hydrophobic and hydrophilic surfaces, causing flat or collapsed drops to appear on the slide.

3.2.4. Molecular Identification of Microorganism

Using 27f/ 519r primers, the identity of the bacterial isolates were determined through amplification and sequencing of its 16s rRNA gene.

3.2.5. Determination of Antibacterial and Antifungal Activity of Biosurfactant

Crude biosurfactant was used to assay for anti-microbial activity using well diffusion method. The crude biosurfactant was tested against *S.aureus ATCC 29213*. two kinds of *E.coli*, *Salmonella spp*., *Klebsiella spp*. and two kinds of *S.aureus* which were isolated from balcali hospital and *S.aureus* which was isolated from the nature for the antimicrobial (antibacterial) activity and *C.albicans* for the antimicrobial (antifungal) activity. For this purpose, stock cultures (100 µL) were inoculated into 5 mL Mueller-Hinton broth and incubated overnight in a shaking incubator at 150 rpm at 30°C. 0.1 mL of the *E.coli 1*, *E.coli 2*, *S.aureus* 1 (isolated from hospital), *S.aureus* 2 (isolated from nature), *S.aureus ATCC 29213*, *Salmonella spp*., *Klebsiella spp* suspensions were taken and cultivated in Mueller Hinton agar, for *C.albicans* stock cultures (100 µL) were inoculated into 5 mL malt extract broth and incubated overnight in a shaking incubator at 150 rpm at 30°C the 0.1 mL of the *C.albicans* was cultivated in Potato Dextrose Agar ,Then sterile empty antibiotic discs were placed in the cultivated agar media (Mueller Hinton agar and Potato Dextrose Agar) after absorbing the pure biosurfactant solution at 25, 50 and 100 µL concentrations and incubated at 30°C for 24-48 hours . At the end of incubation, the zone diameter around the discs was measured. Ampicillin

and penicillin G were used as a positive control in the antibacterial assay and norfloxacin as a positive control in the antifungal assay (Fernandes et all. 2007).

3.2.6. Optimization of Biosurfactant Produced by Isolated Bacteria

3.2.6.1. Effect of pH

For optimization of pH, 3 pH values were selected, 6.0, 7.0 and 8.0. Biosurfactant production Culture medium was prepared by adding 5% olive oil as the sole carbon source and pH was adjusted by pH meter using 6.0 M HCl and 0.1M NaOH solutions. After pH adjustment, the medium was sterilized at 121°C for 15 min. Activated culture of biosurfactant producing bacteria was inoculated. And incubated at 37°C for 3 days in orbital shaker at 150 rpm. Biosurfactant activity were determined by measuring the zone diameters with the oil spreading technique (Parthipan. 2017).

3.2.6.2. Effect of Temperature

For optimization of temperature, 3 temperature values were selected, 30°C, 37°C and 45°C. Biosurfactant production Culture medium was prepared by adding 5% olive oil as the carbon source and pH was adjusted to 7.0 by pH meter using 6.0 M HCl and 0.1M NaOH solutions and sterilized at 121°C for 15 min. Activated culture of biosurfactant producing bacteria was inoculated and incubated at different temperatures for 3 days in orbital shaker at 150 rpm. Biosurfactant activity were determined by measuring the zone diameters with the oil spreading technique (Parthipan. 2017).

3.2.6.3. Effect of Carbon Source

Four carbon sources were used, crude oil, olive oil, sunflower oil and sucrose. Biosurfactant production Culture medium was prepared by adding 5% of the carbon source and pH was adjusted to 7.0 by pH meter using 6.0 M HCl and 0.1M NaOH solutions. And sterilized at 121°C for 15 min. Activated culture of biosurfactant producing bacteria was inoculated and incubated at 37°C for 3 days

in orbital shaker at 150 rpm. Biosurfactant activity were determined by measuring the zone diameters with the oil spreading technique (Parthipan. 2017).

3.2.7. Biosurfactant Extraction

The extraction techniques involve the use of acid precipitation and solvent extraction methods. The fermentation broth sample was centrifuged at 14,000 rpm for 15 min using (J.P selecta, S.A centrifuge) . The obtained supernatant serves as the crude biosurfactant. Crude biosurfactant was treated by acidification to pH 2.0 using 6 M HCl and the acidified supernatant was left overnight at 4°C for complete precipitation of the biosurfactant. Precipitated samples were centrifuged and the pellets obtained serves as the unpurified biosurfactant for acid precipitated samples. Crude biosurfactant was treated with methanol: chloroform (1:2) and centrifuged again to obtain purified biosurfactant precipitate (Tabatabaee. 2005; Batool. 2017; Abu-Ruwaida. 1991). Pure biosurfactant was dried and stored in phosphate buffer.

3.2.8. Structural Characterization of Biosurfactants

3.2.8.1. Fourier Transformation Infra-Red (FTIR) Analysis

FTIR spectrophotometer was used to determine the functional group of crude biosurfactants in aqueous solution (J.H. Unás. 2018)To determine the kind of biosurfactant, the result was compared to numerous resources of FTIR spectrum of various types of biosurfactants.

3.2.8.2. NMR Spectroscopy

The biosurfactant molecule was structurally characterized using 1 H NMR. One-dimensional 1 H nuclear magnetic resonance (NMR) spectrum was recorded on 298K (the number of data points per parts per million of the plot) on a 500 MHz NMR spectrometer (Bruker). The samples were tested at İnönü University, Scientific and Technological Research center, Laboratory of NMR.

4. RESULTS AND DISCUSSION

4.1. Isolation and Identification of Bacterial Strains From Soil Samples

Using 27f/ 519r primers, the identity of the bacterial isolates were determined through amplification and sequencing of its 16s rRNA gene. The 16sRNA gene sequence of the bacteria was aligned with known sequences using the BLAST algorithm in the NCBI database. The bacterium was identified as Bacterial isolate No. 1 is 99% similar to *B.velezensis* (Accession number: NR_116240.1), Bacterial isolate No. 2 is 88.54% similar to *B.haynesii*. (Accession number: NR_157609.1), Bacterial isolate No. 3 is 99.40% similar to *B.halotolerans*. (Accession number: NR_115931.1), Bacterial isolate No. 4 is 99.29% similar to *P.reinekei*. (Accession number: NR_042541.1), Bacterial isolate No. 5 is 99.34% similar to *M.arborescens*. (Accession number: NR_029265.1), Bacterial isolate No. 6 is 98.97% similar to *P.veronii*. (Accession number: NR_028706.1) and Bacterial isolate No. 7 is 99.14% similar to *A.lwoffii*. (Accession number: NR_113346.1).

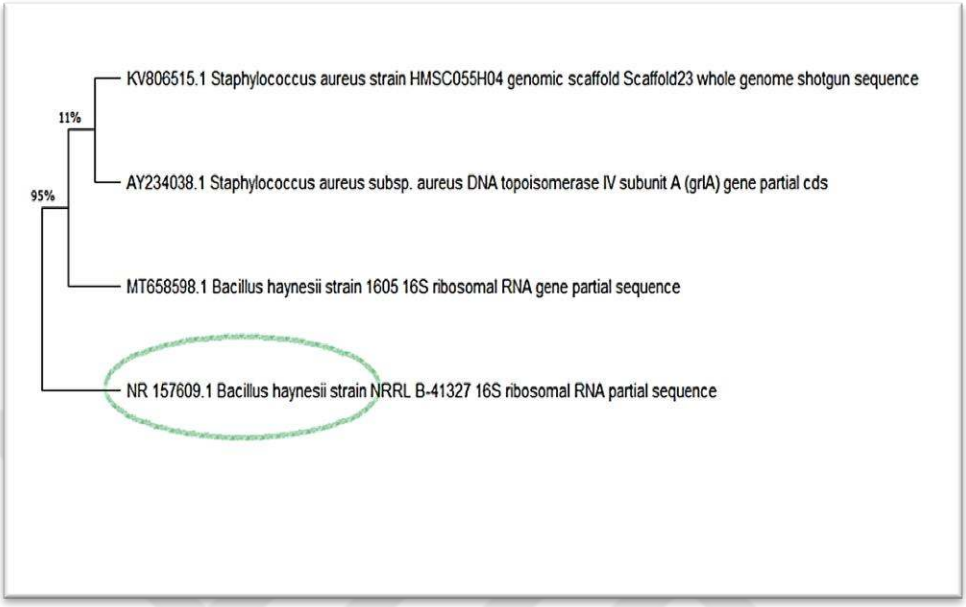


Figure 4.1. The phylogenetic tree of the isolated microrganism

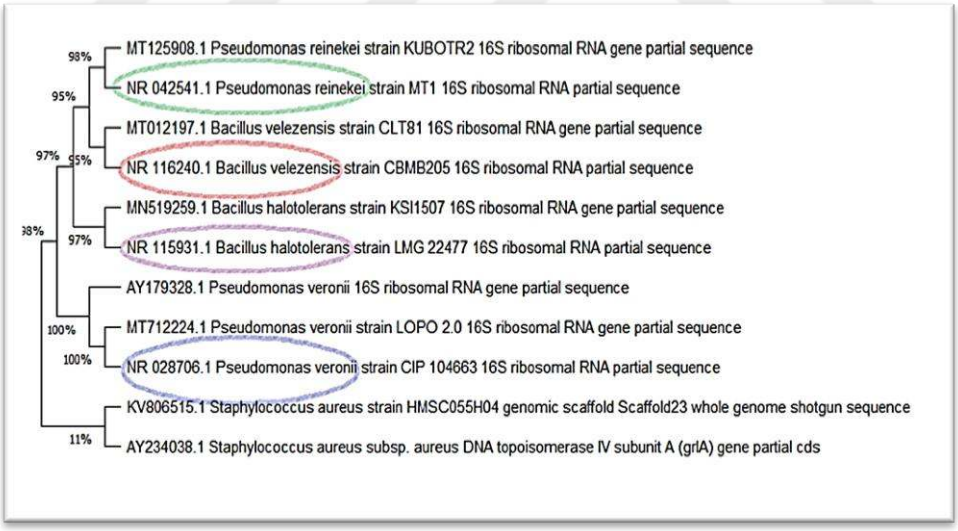


Figure 4.2. The phylogenetic tree of the isolated microrganism

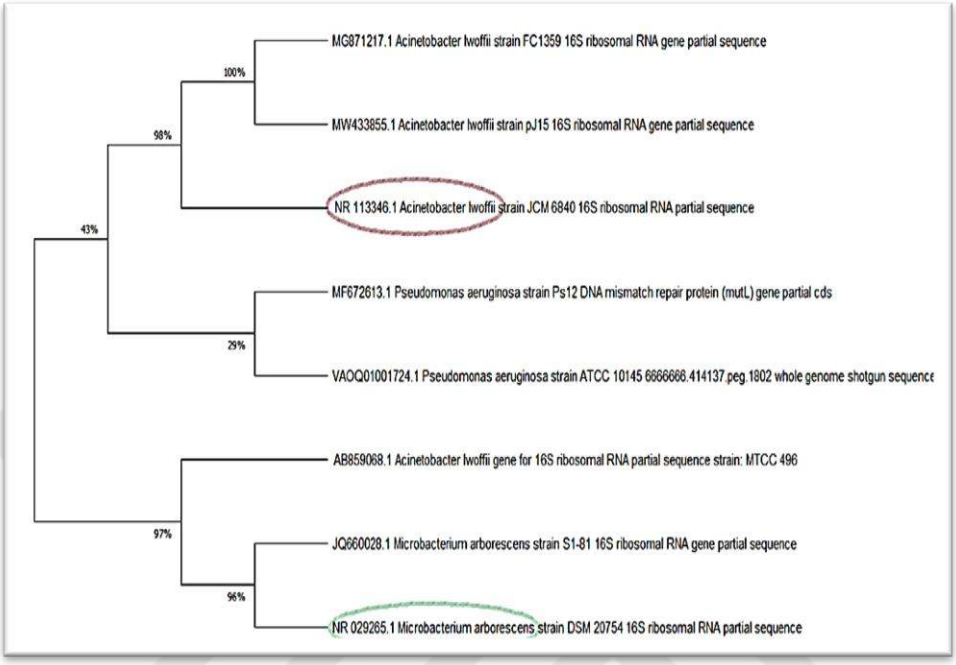


Figure 4.3. The phylogenetic tree of the isolated microrganism

4.2. Biosurfactant Production Culture Medium

A mineral salt medium with the following content (g/L) was used for biosurfactant production: 2.5 g/L $NaNO_3$, 0.1 g/L KCL, 3.0 g/L KH_2PO_4, 7.0 g/L K_2HPO_4, 0.01 g/L CaCl2, 0.5 g/L $MgSO_4.7H_2O$) supplemented with 5 mL trace element solution (0.116 g/L of $FeSO_4.7H_2O$, 0.232 g/L of H_3BO_3, 0.41 g/L of $CoCl_2.6H_2O$, 0.008 g/L of $CuSO_4.5H_2O$, 0.008 g/ L of $MnSO_4.H_2O$, 0.022 g/L of [NH4]6Mo_7O_{24}, 0.174 g/L of $ZnSO_4$) , then supplemented with 5% olive oil as substrate and autoclaved for 15 minutes at 121°C , the bacterial sample in nutrient agar medium was inoculated from the solid medium into 10 mL Nutrient broth for resuscitation and incubated in a shaking incubator at 150 rpm for 1 night at 37ºC.After the incubation, the bacterial culture was inoculated at a rate of 10% in 100 mL medium (Biosurfactant production Culture medium), pH 7.0 and incubated for 3 days at 37°C and 150 rpm.

4.3. Screening of Biosurfactant Producing Isolates

The key of success to find new biosurfactant producers is an efficient screening strategy. Screening of the bacteria obtained from the soil for biosurfactant production, only strains that gave positive result for all tests were selected as producers.

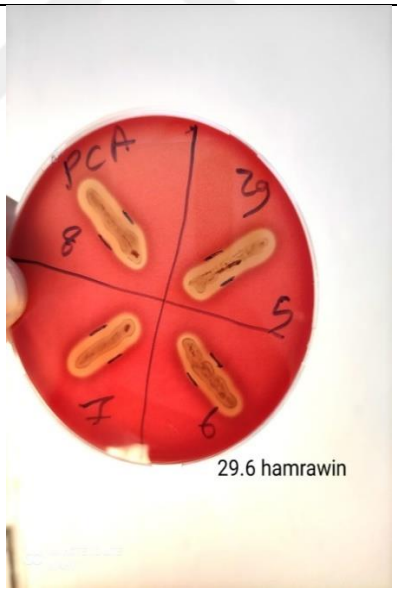
4.3.1. Haemolytic Activity

4.3.1.1. Haemolytic Activity of The Biosurfactant-Producing Bacteria

Fresh colonies were taken and smeared on blood agar plates containing 7 % v/v human blood. These plates were incubated at 37°C for 1-7 days The plates were then observed every day for the presence of clear zone around the colonies which indicating that biosurfactant-producing organisms were present. Alpha haemolysis or partial haemolysis is indicated by the appearance of a partial transparent zones beneath the colonies. Beta or complete haemolysis of the blood cells is indicated by a yellow transparent zone around the colony. Gamma or no haemolysis is shown by no change in the blood agar plates. Amongst more than 50 isolates showed positive result for haemolytic activity and we selected 10 isolates.

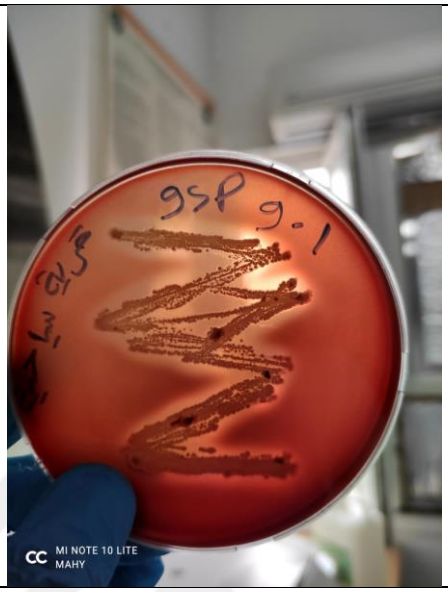
Figure 4.4. Alpha and Beta haemolysis of the blood cells

Table 4.1. Haemolytic Activity of the isolated biosurfactant-producing bacteria.

microorganism	Incubation time/ Diameter of the clear zone		Haemo lysis type	Figure
B.velezensis	24 h	0.45cm	Beta	
	48 h	0.5cm		
	72 h	0.9cm		
B.halotolerans	24 h	0.85cm	Beta	
	48 h	1.1cm		
	72 h	1.3cm		

B.haynesii	24 h	1.0cm	Alpha	
	48 h	1.05cm		
	72 h	1.1cm		
M.arborescens	24 h	-	Beta	
	48 h	-		
	72 h	-		
	96 h	2.0cm		
	120 h	3.4cm		

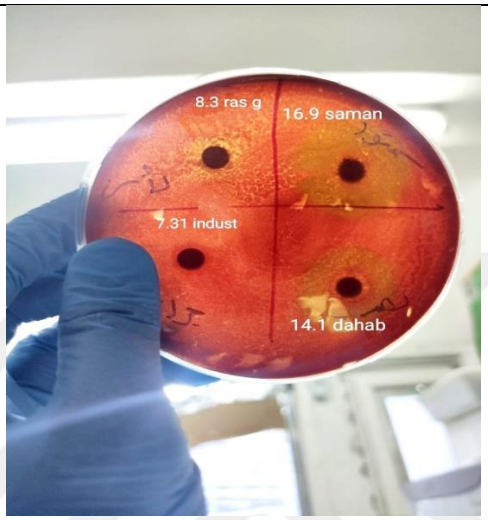
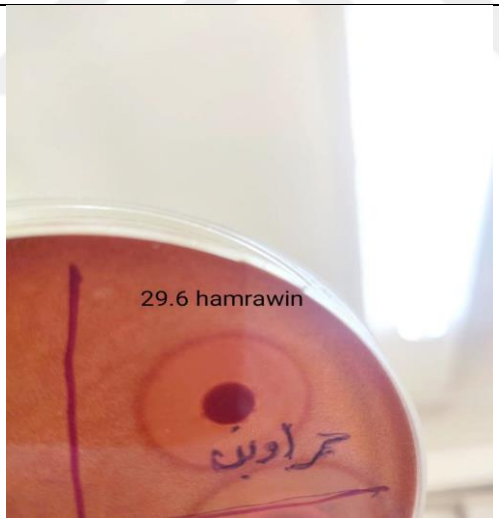
P.veronii	24 h	-	Beta	
	48 h	-		
	72 h	-		
	96 h	1.8cm		
	120 h	2.5cm		
P.reinekei	24 h	-	Beta	
	48 h	-		
	72 h	-		
	96 h	1.9cm		
	120 h	2.6cm		

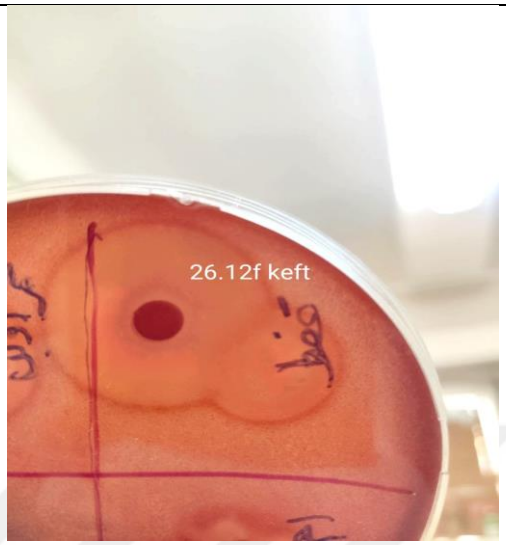
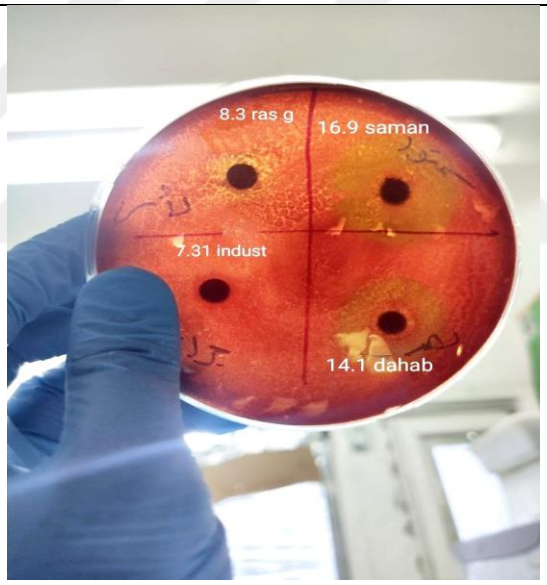
A.lwoffii	24 h	-	Alpha	
	48 h	-		
	72 h	1.0cm		
	96 h	1.0cm		

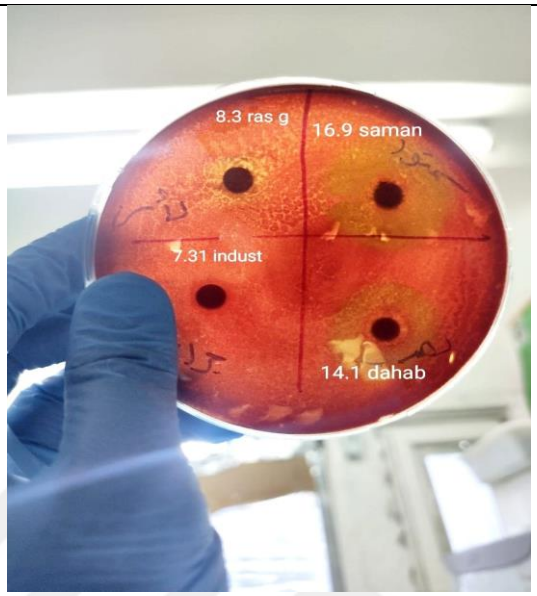
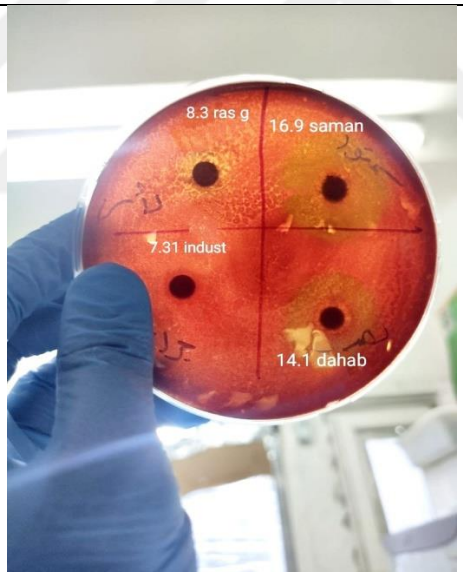
4.3.1.2. Haemolytic Activity of The Crude Biosurfactant Solution

Blood agar media containing 7 % v/v human blood was inoculated with pure biosurfactant solution, sterile empty antibiotic discs were placed in the Blood agar media after absorbing the pure biosurfactant solution at 50 µL concentration and incubated at 37°C for 24 hours. The plates were then observed for the presence of clear zone around the discs. Alpha haemolysis (partial) is indicated by a partial transparent zone beneath the colonies. Beta (complete) of the blood cells is indicated by a yellow transparent zone around the colony. Gamma or no haemolysis is shown by no change in the blood agar plates. The zone diameter around the discs was measured.

Table 4.2. Haemolytic Activity of the pure biosurfactant solution.

microorganism	Diameter of the clear zone	Figure
B.velezensis	2.2 cm	
B.halotolerans	2.0 cm	

B.haynesii	2.8 cm	
P.reinekei	2.8 cm	

M.arborescens	No haemolysis	
P.veronii	1.0 cm	

4.3.2. Surface Tension

The surface tension of the cell free culture broth obtained after 24 h, 48 h, 72 h and 15 days of incubation was measured by using the Optical Contact Angle / Surface Tension Measurement Device (TDIC - LAUDA) and the Ring method.

Figure 4.5. Surface tension measurement by using the Optical Contact Angle / Surface Tension Measurement Device (TDIC - LAUDA) and the Ring method

Table 4.3. The surface tension of the isolated biosurfactant-producing bacteria

microorganism	Incubation time /Surface tension (mN/m)				
	0 h	24 h	48 h	72 h	15 days
B.velezensis	50.0	39.4	38.2	36.1	36.7
B.halotolerans	50.0	40.9	39.6	36.5	41.0
B.haynesii	50.0	48.2	47.4	42.5	46.4
P.reinekei	50.0	49.6	48.0	47.1	43.3
M.arborescens	50.0	48.7	47.8	46.3	44.7
P.veronii	50.0	48.0	46.2	44.7	43.6
A.lwoffii	50.0	49.7	46.2	43.8	43.3

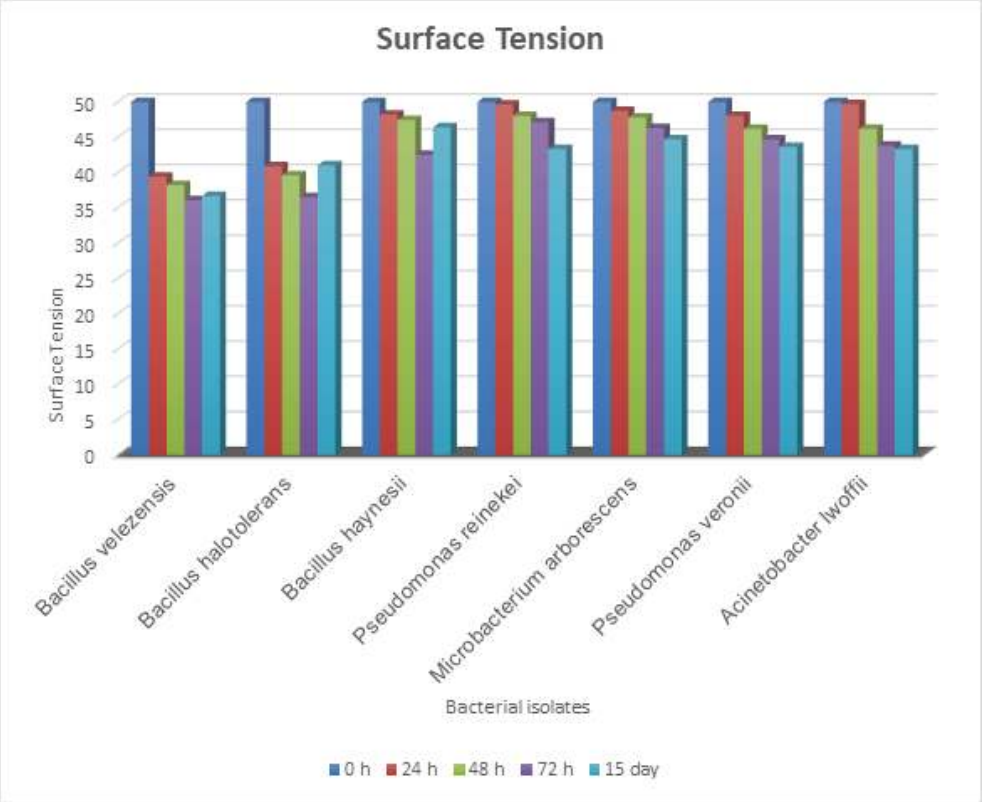
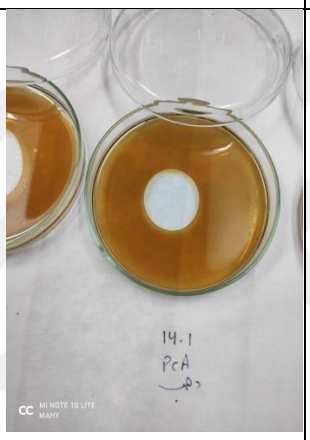
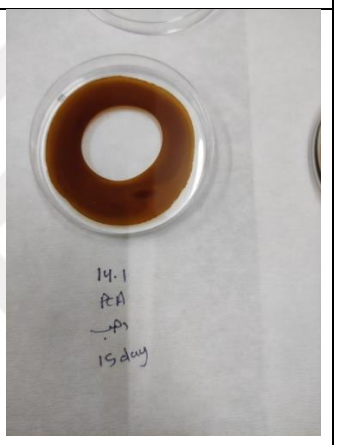
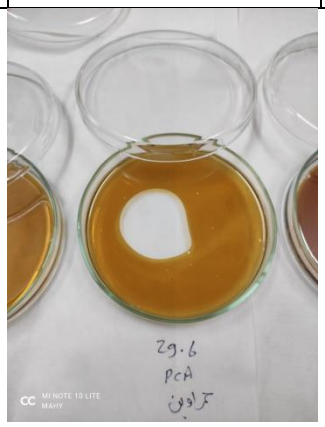
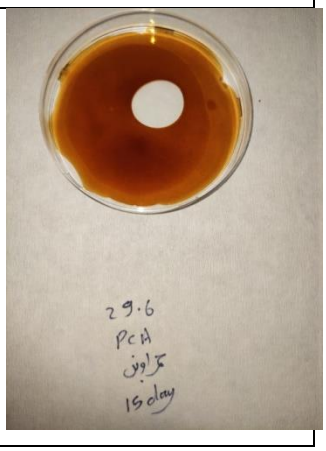


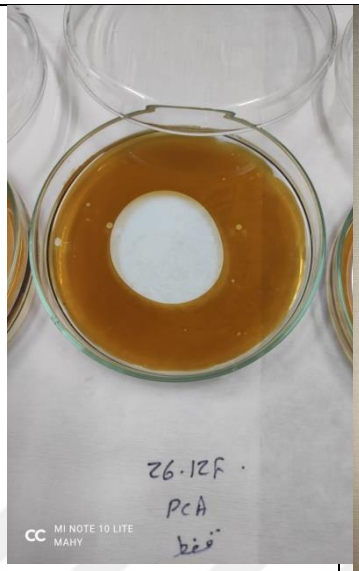
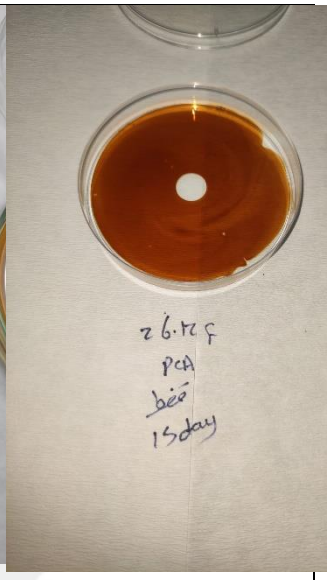
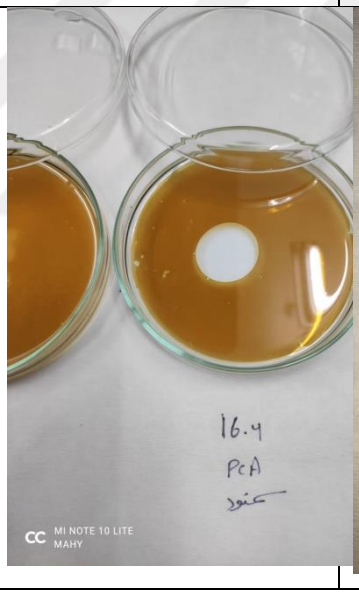
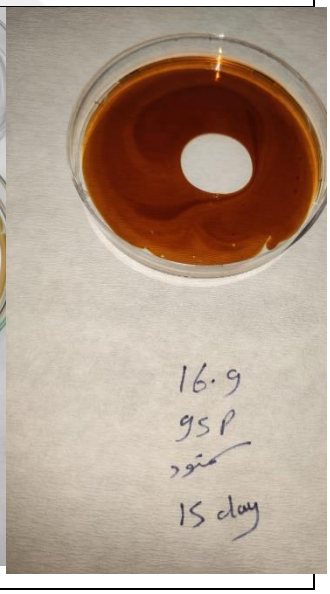
Figure 4.6. The surface tension (mN/m) of the biosurfactant-producing bacteria

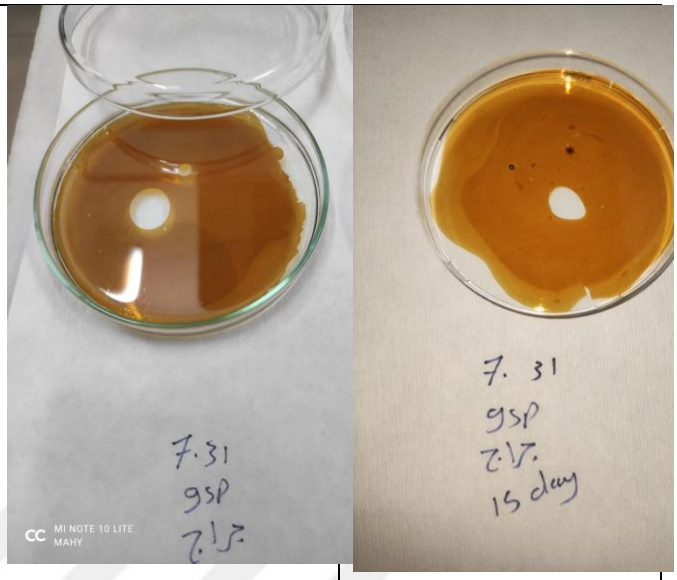
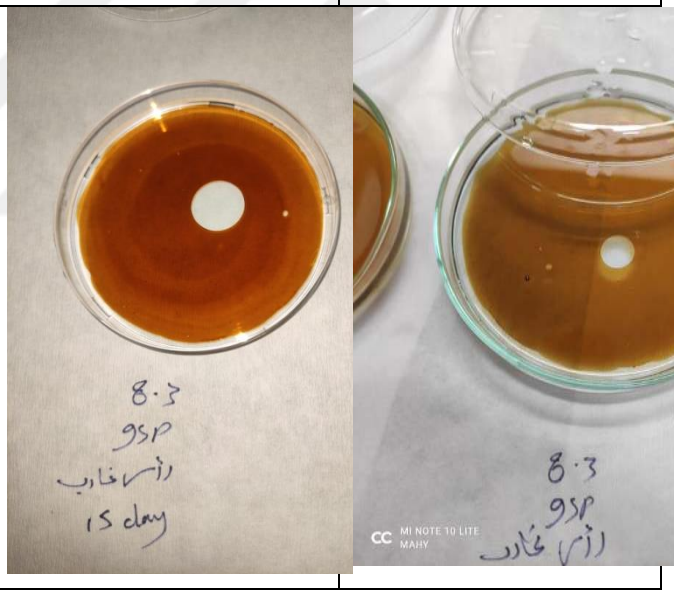
4.3.3. Oil Spread Technique

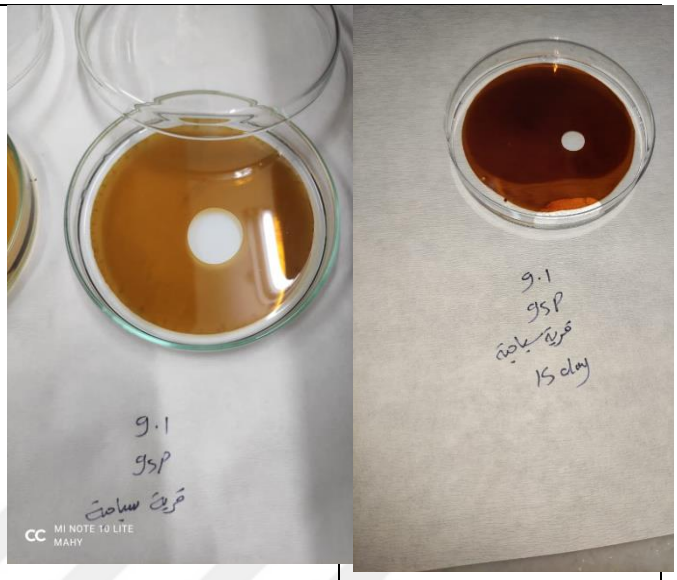
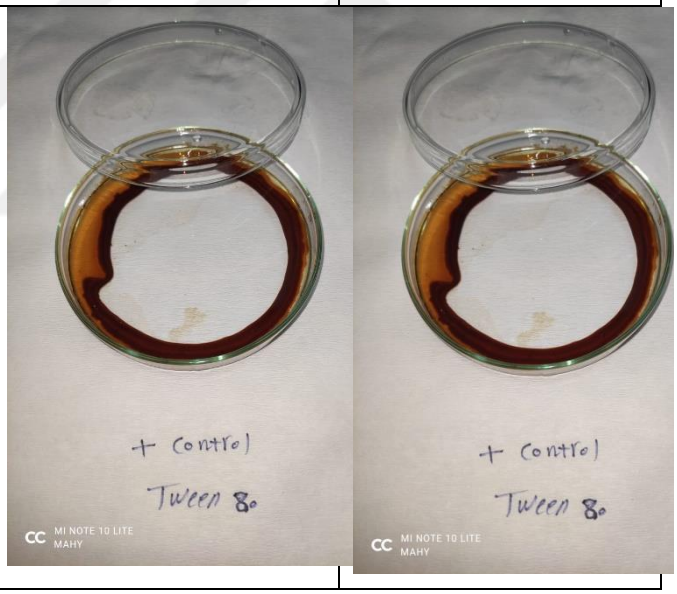
30 mL of distilled water was added to a petri dish of 90 mm diameter followed by addition of 100 µL of crude oil to the surface of the water. 20 µL of cell free culture broth was then added to the oil surface. If biosurfactant is present in the cell-free culture broth, the oil will be displaced by an oil-free clearing zone, the diameter of the oil-free clearing zone was measured. The negative control was distilled water, in which no oil displacement or clean zone was seen, while the positive control was tween 80.

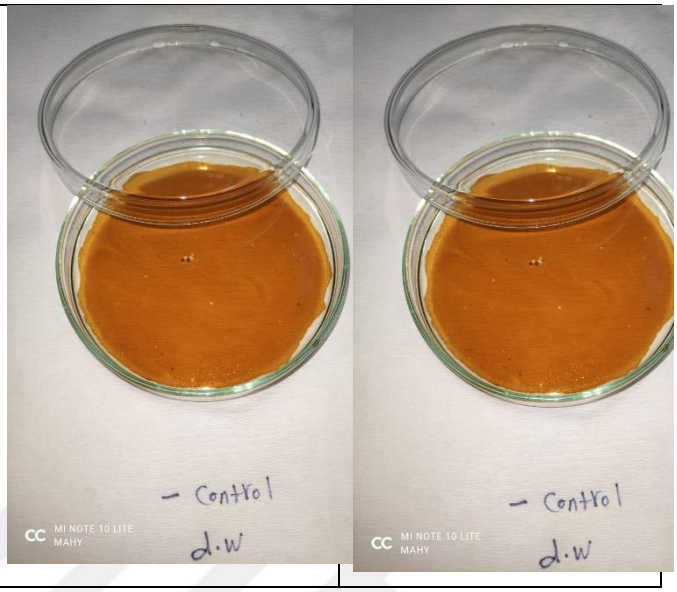
Table 4.4. Oil spread technique of the isolated biosurfactant-producing bacteria

microorganism	Incubation time/ oil-free clearing zone diameter		Figure	
	72 h	15 days	72 h	15 days
B.velezensis	4.0 cm	5.8 cm		
B.halotolerans	5.0 cm	3.4 cm		

B.haynesii	4.6 cm	1.7 cm		
P.reinekei	4.0 cm	4.0 cm		

M.arborescens	2.1 cm	1.4 cm	
P.veronii	2.2 cm	2.0 cm	

A.lwoffii	2.7 cm	1.4 cm	
positive control (tween 80)	8.0 cm	8.0 cm	

negative control (distilled water)	-	-	

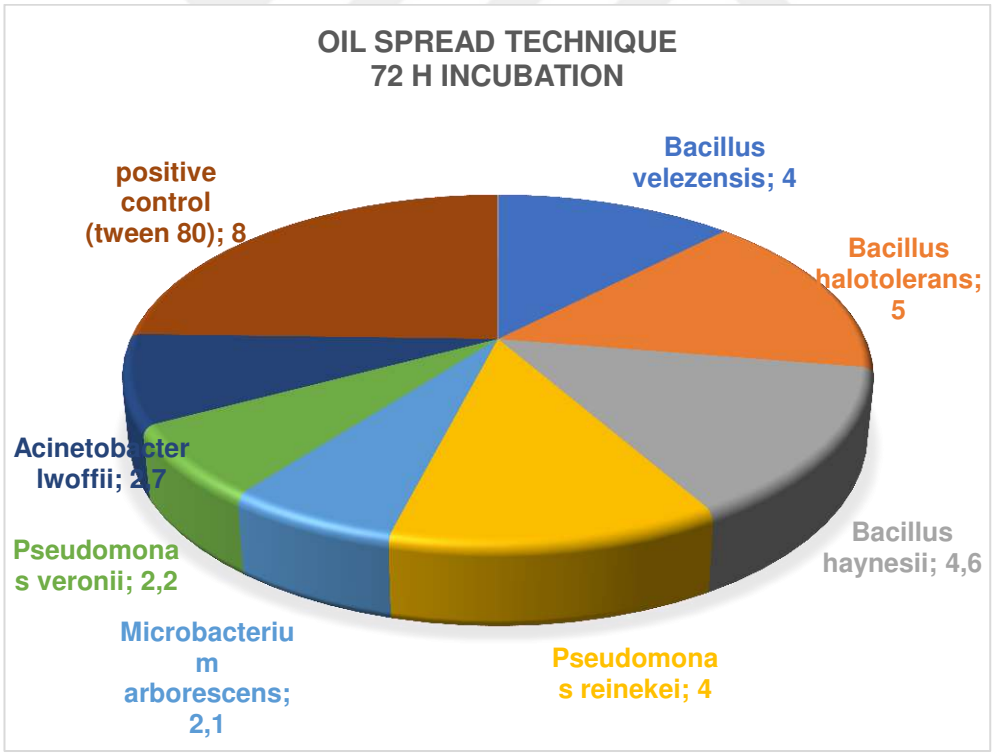


Figure 4.7. Oil spread technique of the biosurfactant-producing bacteria (72 h incubation)

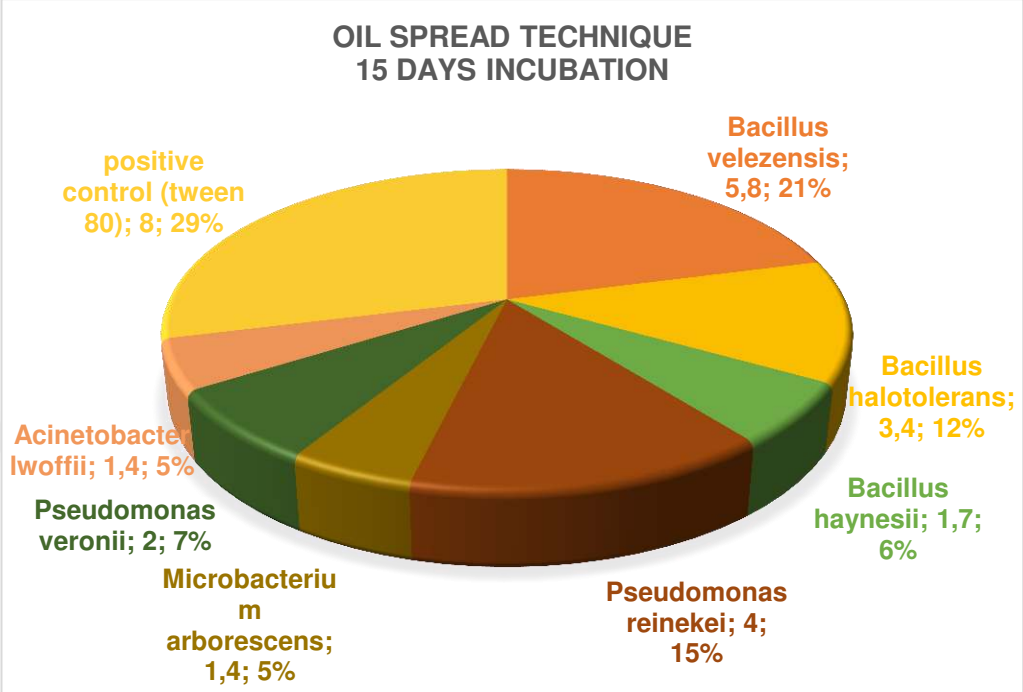


Figure 4.8. Oil spread technique of the biosurfactant-producing bacteria (15 days incubation)

4.3.4. Emulsification Activity

Emulsification index (E24) of the biosurfactant towards Hydrocarbons such as xylene, n-heptane and sunflower oil was done. A mixture of 2 mL hydrocarbon and 2 mL cell free extract obtained after the centrifugation of sample culture were taken in a test tube and homogenized by vortexing at high speed for 2 min. The emulsion activity was investigated after 1 h, 24 h, 48h, 72h, 96h, 120h, 144h, 168h and 192h. The emulsification index (E24) was calculated using following formula: E24 (%) = total height of the emulsified layer/total height of the liquid layer × 100.The results were compared with tween 80 as (+ve) control and distilled water as a (-ve) control.

Figure 4.9. Emulsification activity using n- heptan as hydrocarbon source

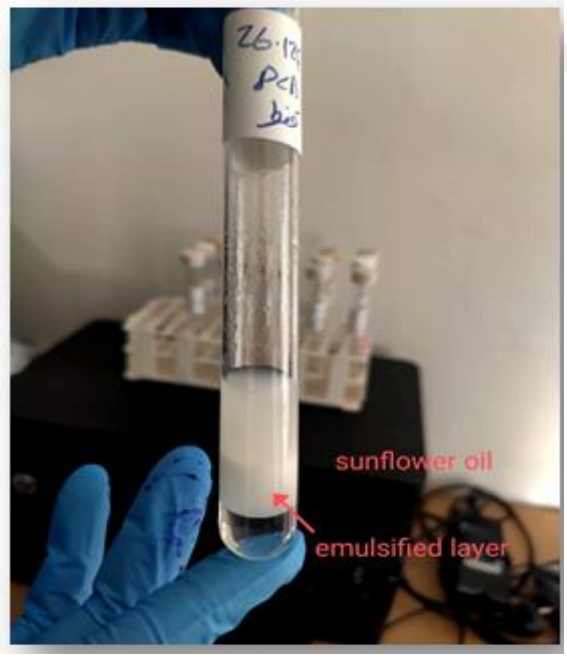


Figure 4.10. Emulsification activity using sunflower oil as hydrocarbon source

Figure 4.11. Emulsification activity using xylene as hydrocarbon source

Table 4.5. Emulsification index values of biosurfactant of *B.velezensis* on various hydrocarbons using pure biosurfactant solution incubated at 37°C for 72 hours.

Time	Emulsification index (E24) %		
	xylene	sunflower oil	n-heptane
1 h	47.6	35.7	4.8
24 h	36.9	20.2	3.6
48 h	26.2	14.3	2.4
72 h	23.8	14.3	2.4
96 h	20.2	14.3	2.4
120 h	14.3	11.9	-
144 h	14.3	9.5	-
168 h	11.9	9.5	-
192 h	9.5	9.5	-

Table 4.6. Emulsification index values of biosurfactant of *B.velezensis* on various hydrocarbons using pure biosurfactant solution incubated at 37°C for 15 days.

Time	Emulsification index (E24) %		
	xylene	sunflower oil	n-heptane
1 h	9.5	39.0	23.8
24 h	2.4	19.5	9.5
48 h	2.4	9.8	2.4
72 h	-	7.3	2.4
96 h	-	7.3	2.4
120 h	-	6.1	2.4
144 h	-	4.9	2.4
168 h	-	4.9	2.4
192 h	-	4.9	2.4

Table 4.7. Emulsification index values of biosurfactant of *B.halotolerans* on various hydrocarbons using pure biosurfactant solution incubated at 37°C for 72 hours.

Time	Emulsification index (E24) %		
	xylene	sunflower oil	n-heptane
1 h	19	28.6	5.0
24 h	14.3	7.1	2.5
48 h	7.1	4.8	2.5
72 h	2.4	4.8	2.5
96 h	2.4	3.6	2.5
120 h	2.4	3.6	2.5
144 h	2.4	3.6	2.5
168 h	2.4	3.6	2.5
192 h	2.4	3.6	2.5

Table 4.8. Emulsification index values of biosurfactant of *B.halotolerans* on various hydrocarbons using pure biosurfactant solution incubated at 37°C for 15 days.

Time	Emulsification index (E24) %		
	xylene	sunflower oil	n-heptane
1 h	11.9	37.0	5.8
24 h	2.4	14.0	3.5
48 h	2.4	9.3	3.5
72 h	-	9.3	3.5
96 h	-	9.3	3.5
120 h	-	9.3	3.5
144 h	-	9.3	3.5
168 h	-	9.3	3.5
192 h	-	9.3	3.5

Table 4.9. Emulsification index values of biosurfactant of *B.haynesii* on various hydrocarbons using pure biosurfactant solution incubated at 37°C for 72 hours.

Time	Emulsification index (E24) %		
	xylene	sunflower oil	n-heptane
1 h	33.3	34.1	6.0
24 h	27.4	19.5	3.7
48 h	26.2	14.6	3.7
72 h	23.8	14.6	2.4
96 h	23.8	14.6	2.4
120 h	23.8	14.6	2.4
144 h	23.8	14.6	2.4
168 h	23.8	14.6	2.4
192 h	23.8	14.6	2.4

Table 4.10. Emulsification index values of biosurfactant of *B.haynesii* on various hydrocarbons using pure biosurfactant solution incubated at 37°C for 15 days.

Time	Emulsification index (E24) %		
	xylene	sunflower oil	n-heptane
1 h	38.1	29.3	2.4
24 h	-	12.2	2.4
48 h	-	12.2	2.4
72 h	-	12.2	2.4
96 h	-	12.2	2.4
120 h	-	12.2	2.4
144 h	-	12.2	2.4
168 h	-	12.2	2.4
192 h	-	12.2	2.4

Table 4.11. Emulsification index values of biosurfactant of *P.reinekei* on various hydrocarbons using pure biosurfactant solution incubated at 37°C for 72 hours.

Time	Emulsification index (E24) %		
	xylene	sunflower oil	n-heptane
1 h	23.3	38.1	2.4
24 h	16.3	9.5	2.4
48 h	9.3	7.1	2.4
72 h	9.3	7.1	2.4
96 h	9.3	7.1	-
120 h	9.3	7.1	-
144 h	9.3	7.1	-
168 h	9.3	7.1	-
192 h	9.3	7.1	-

Table 4.12. Emulsification index values of biosurfactant of *P.reinekei* on various hydrocarbons using pure biosurfactant solution incubated at 37°C for 15 days.

Time	Emulsification index (E24) %		
	xylene	sunflower oil	n-heptane
1 h	11.9	29.3	30.0
24 h	11.9	2.4	20.0
48 h	11.9	2.4	20.0
72 h	11.9	2.4	18.8
96 h	11.9	2.4	18.8
120 h	11.9	2.4	18.8
144 h	11.9	1.8	18.8
168 h	10.7	1.8	18.8
192 h	10.7	1.8	18.8

Table 4.13. Emulsification index values of biosurfactant of *M.arborescens* on various hydrocarbons using pure biosurfactant solution incubated at 37°C for 72 hours.

Time	Emulsification index (E24) %		
	xylene	sunflower oil	n-heptane
1 h	12.2	24.4	2.4
24 h	12.2	12.2	2.4
48 h	12.2	9.8	2.4
72 h	12.2	9.8	2.4
96 h	12.2	9.8	-
120 h	9.8	8.5	-
144 h	8.5	7.3	-
168 h	7.3	7.3	-
192 h	7.3	7.3	-

Table 4.14. Emulsification index values of biosurfactant of *M.arborescens* on various hydrocarbons using pure biosurfactant solution incubated at 37°C for 15 days.

Time	Emulsification index (E24) %		
	xylene	sunflower oil	n-heptane
1 h	9.5	17.9	2.5
24 h	-	2.6	2.5
48 h	-	2.6	2.5
72 h	-	2.6	2.5
96 h	-	2.6	2.5
120 h	-	2.6	2.5
144 h	-	2.6	2.5
168 h	-	2.6	2.5
192 h	-	2.6	2.5

Table 4.15. Emulsification index values of biosurfactant of *P.veronii* on various hydrocarbons using pure biosurfactant solution incubated at 37°C for 72 hours.

Time	Emulsification index (E24) %		
	xylene	sunflower oil	n-heptane
1 h	61.0	36.5	12.2
24 h	61.0	7.3	9.8
48 h	61.0	4.9	9.8
72 h	14.6	3.7	9.8
96 h	14.6	3.7	9.8
120 h	12.2	2.4	9.8
144 h	11.0	2.4	9.8
168 h	5.0	2.4	9.8
192 h	5.0	2.4	9.8

Table 4.16. Emulsification index values of biosurfactant of *P.veronii* on various hydrocarbons using pure biosurfactant solution incubated at 37°C for 15 days.

Time	Emulsification index (E24) %		
	xylene	sunflower oil	n-heptane
1 h	28.6	23.1	2.6
24 h	19.0	5.1	2.6
48 h	8.3	5.1	2.6
72 h	2.4	3.8	2.6
96 h	2.4	3.8	2.6
120 h	-	3.8	-
144 h	-	3.8	-
168 h	-	3.8	-
192 h	-	3.8	-

Table 4.17. Emulsification index values of biosurfactant of *A.lwoffii* on various hydrocarbons using pure biosurfactant solution incubated at 37°C for 72 hours.

Time	Emulsification index (E24) %		
	xylene	sunflower oil	n-heptane
1 h	21.4	17.1	4.9
24 h	9.5	2.4	2.4
48 h	8.3	-	2.4
72 h	-	-	2.4
96 h	-	-	-
120 h	-	-	-
144 h	-	-	-
168 h	-	-	-
192 h	-	-	-

Table 4.18. Emulsification index values of biosurfactant of *A.lwoffii* on various hydrocarbons using pure biosurfactant solution incubated at 37°C for 15 days.

Time	Emulsification index (E24) %		
	xylene	sunflower oil	n-heptane
1 h	11.9	33.3	3.8
24 h	-	-	2.6
48 h	-	-	2.6
72 h	-	-	2.6
96 h	-	-	-
120 h	-	-	-
144 h	-	-	-
168 h	-	-	-
192 h	-	-	-

Table 4.19. Emulsification index values of positive control (tween 80) on various hydrocarbons.

Time	Emulsification index (E24) %		
	xylene	sunflower oil	n-heptane
1 h	100.0	88.5	82.5
24 h	100.0	52.5	77.5
48 h	93.0	52.5	77.5
72 h	90.7	52.5	77.5
96 h	88.4	52.5	77.5
120 h	86.0	52.5	77.5
144 h	86.0	52.5	77.5
168 h	86.0	52.5	77.5
192 h	86.0	52.5	77.5

Table 4.20. Emulsification index values of negative control (distilled water) on various hydrocarbons.

Time	Emulsification index (E24) %		
	xylene	sunflower oil	n-heptane
1 h	-	-	-

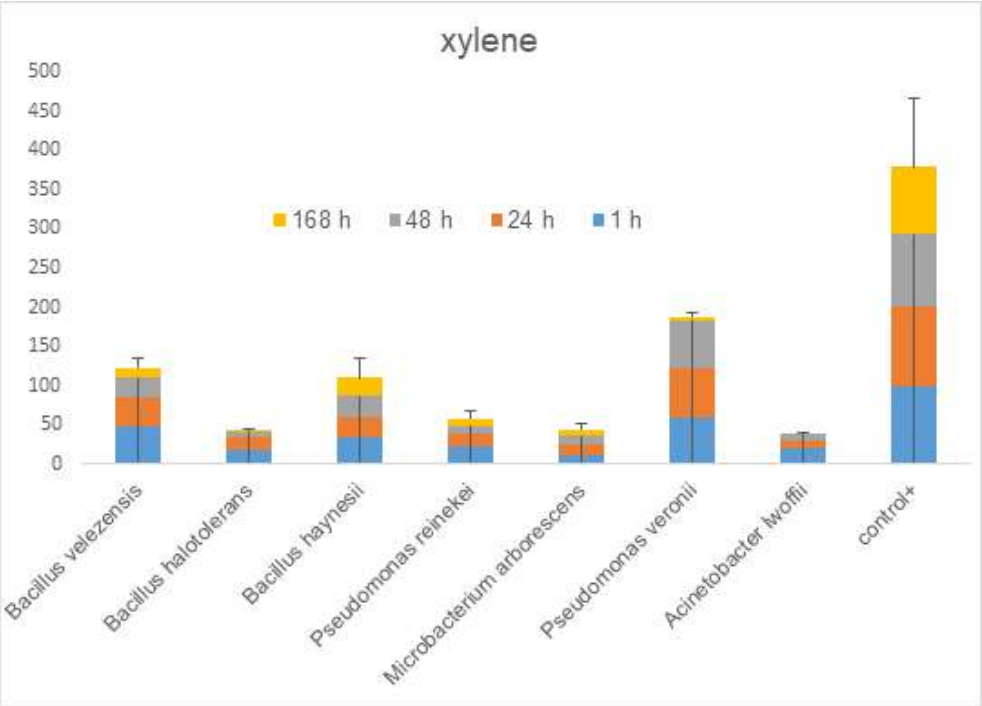


Figure 4.12. Emulsification index with xylene

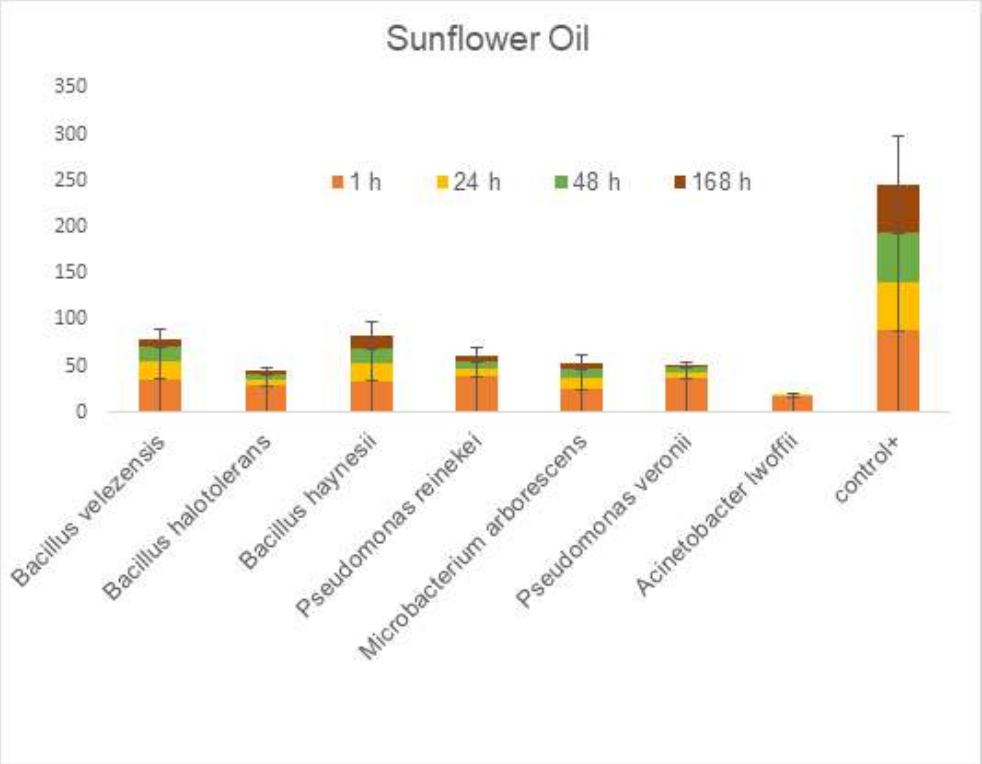


Figure 4.13. Emulsification index with sunflower oil

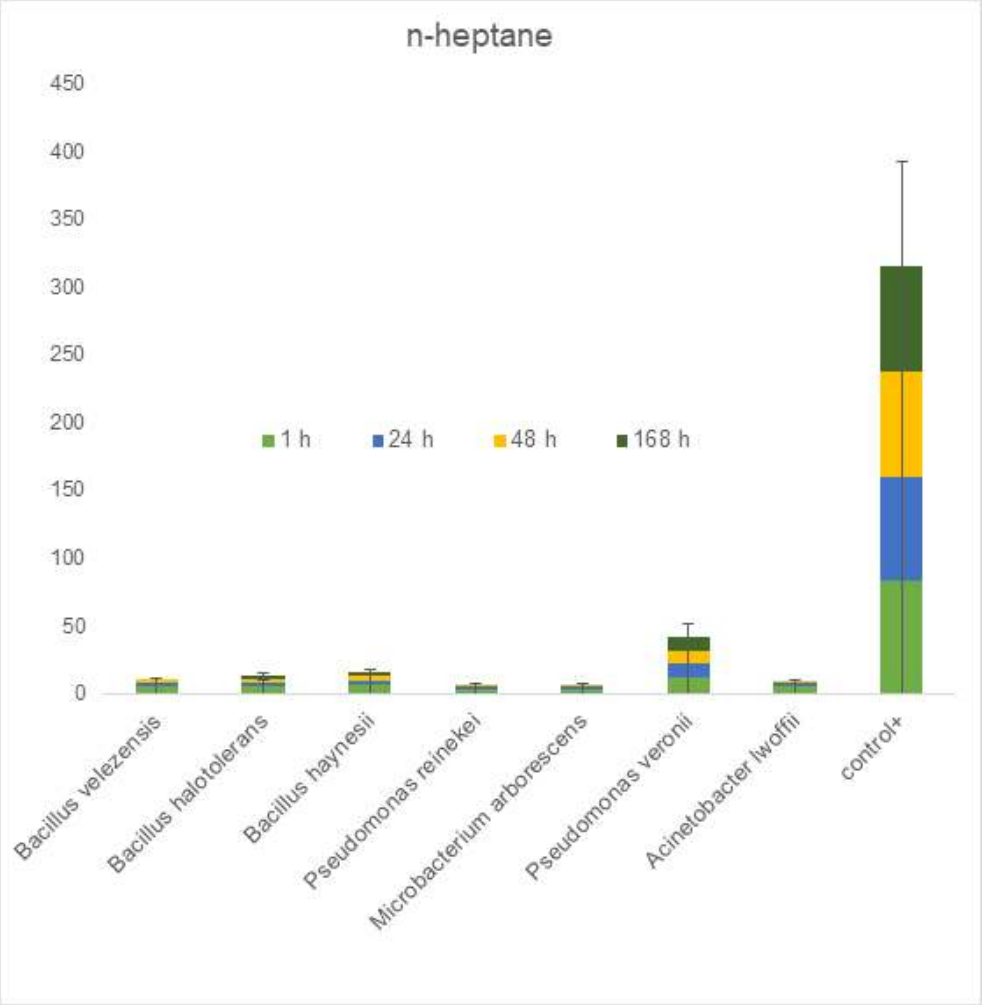


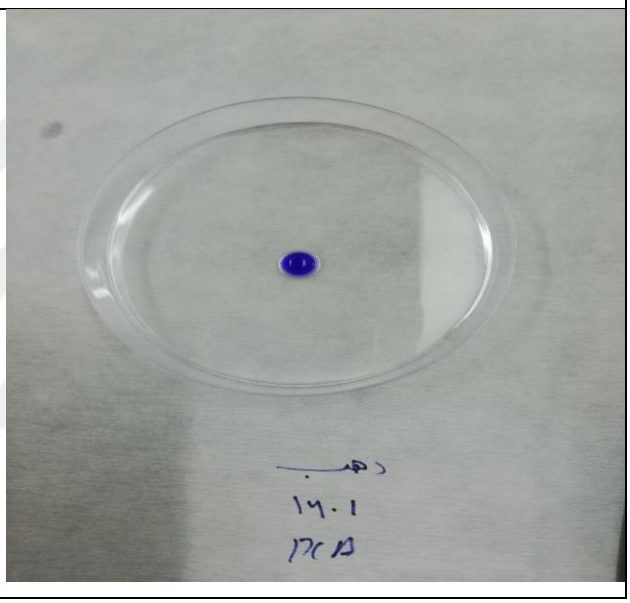
Figure 4.14. Emulsification index with n-heptane

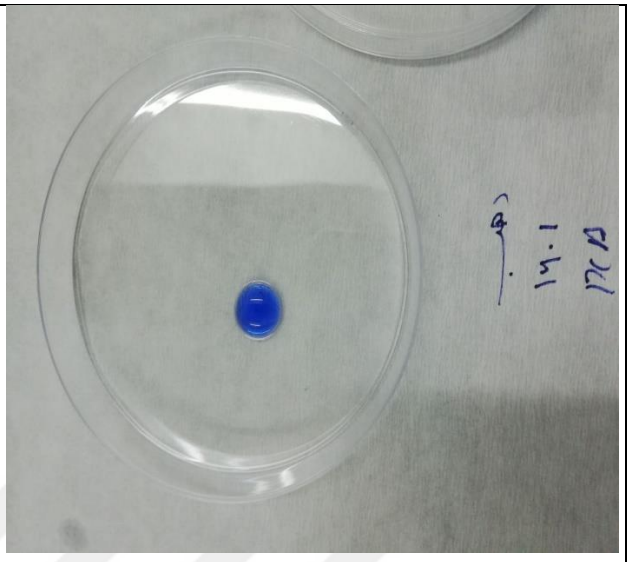
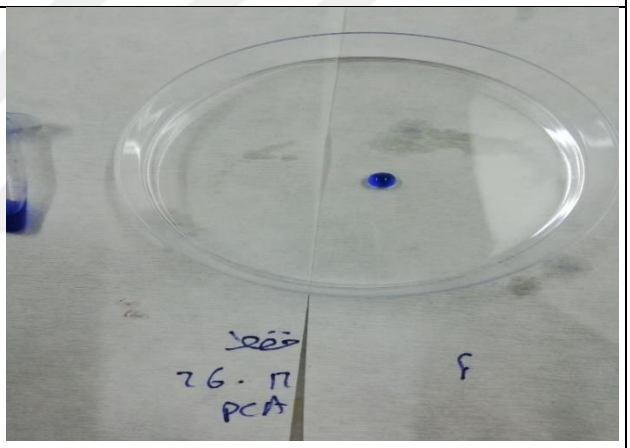
4.3.5. Drop Collapse Assay

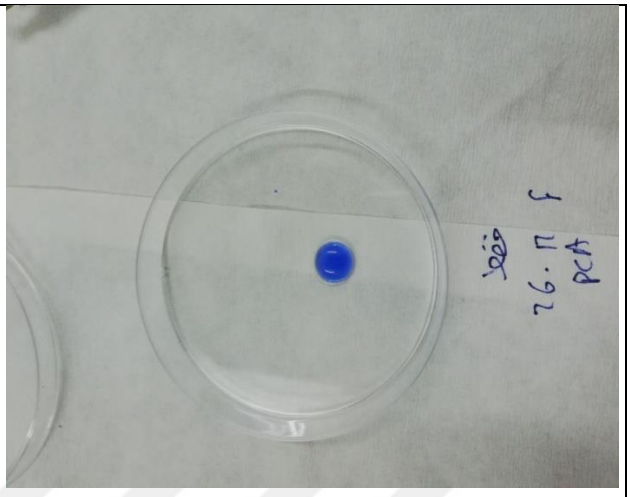
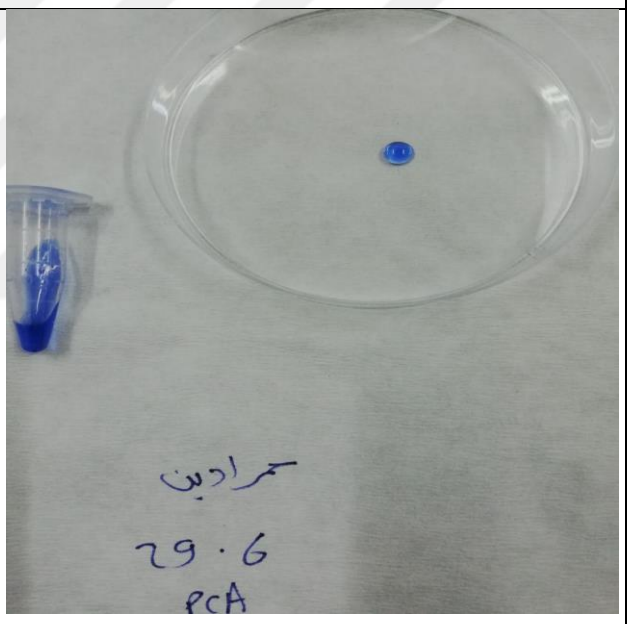
On a clean glass petri dish, a single droplet of oil and supernatant was placed. After 1 minute, the shape of the drop on the oil surface was examined. The presence of surfactant in the culture supernatant causes the drop to collapse, the diameter of the oil drop is measured before adding the supernatant and after 1 min. Tween 80 was utilized as (+ve) control and distilled water as (-ve) control.

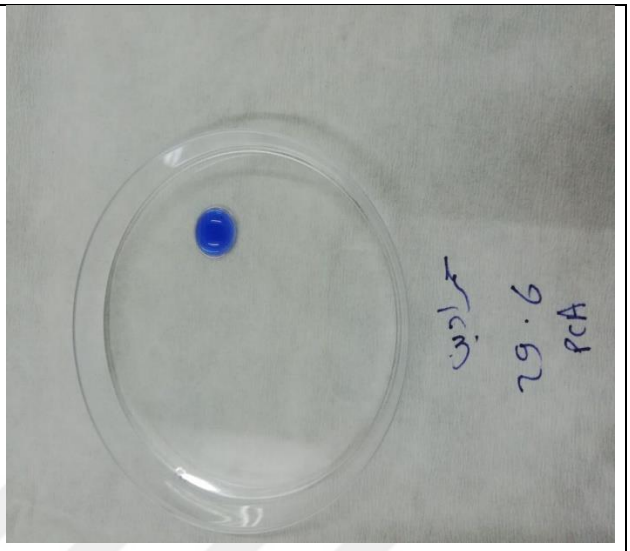
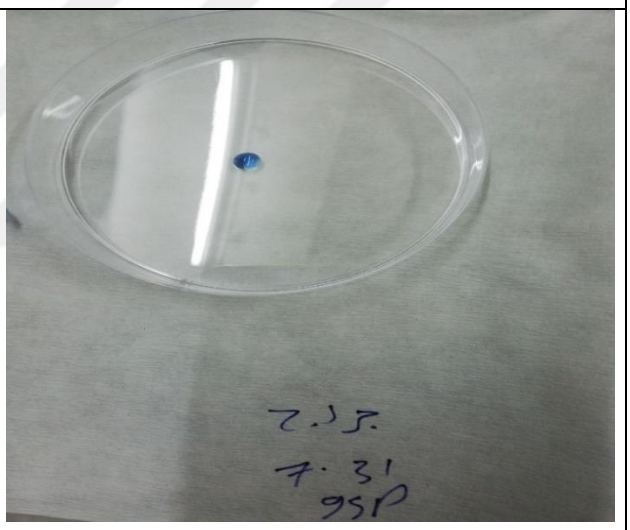
methylene blue is added to the oil to have a clearer vision when the collapse occurred.

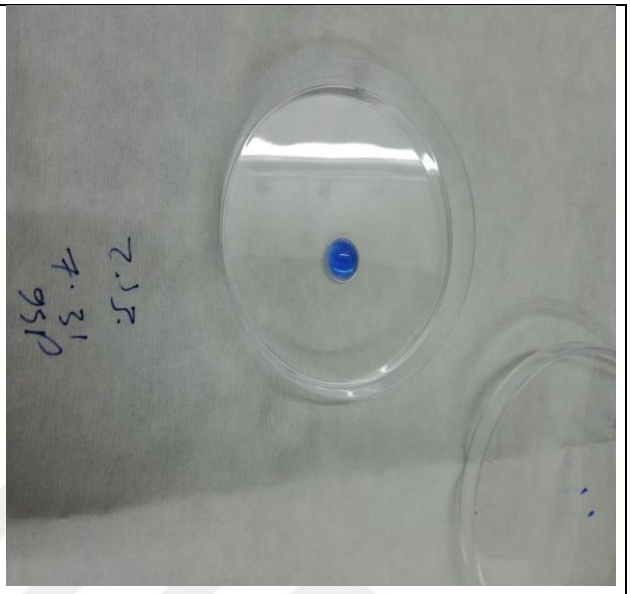
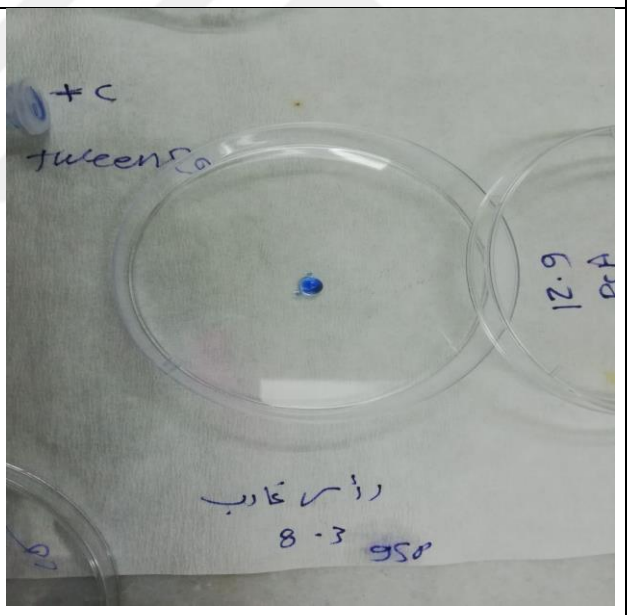
Table 4.21. Drop collapse assay of the isolated biosurfactant-producing bacteria

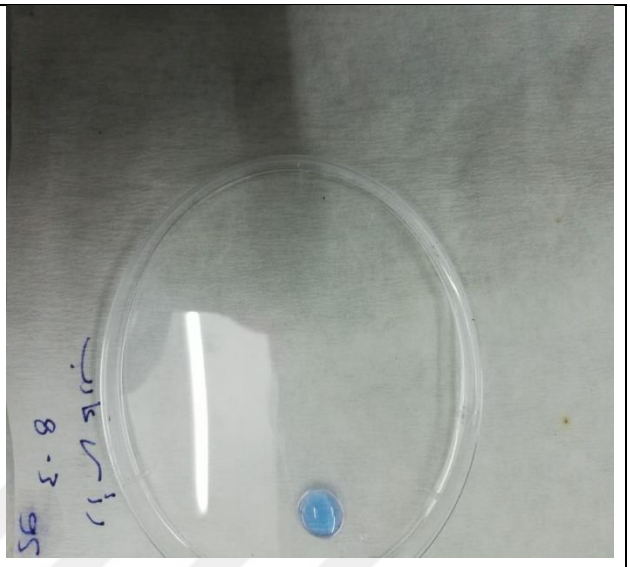
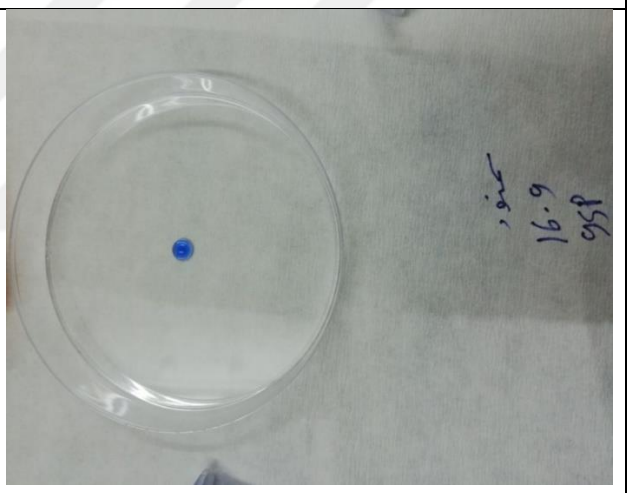
microorganism	The diameter of the oil drop		Figure
B.velezensis	0 min	0.5 cm	

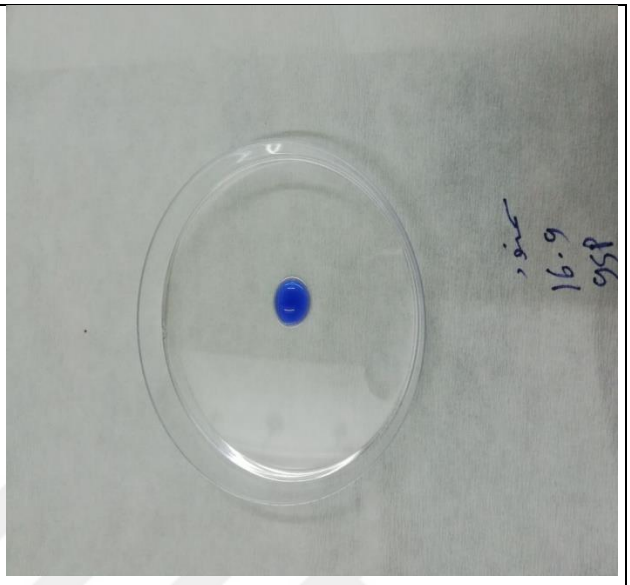
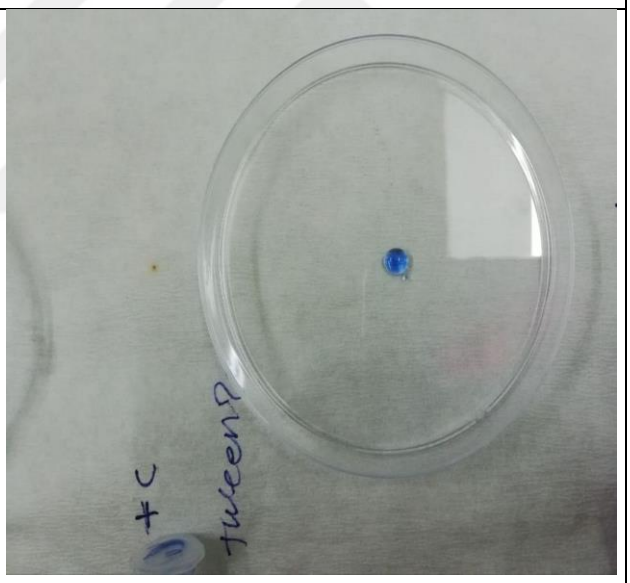
	1 min	0.6 cm	
B.haynesii	0 min	0.5 cm	

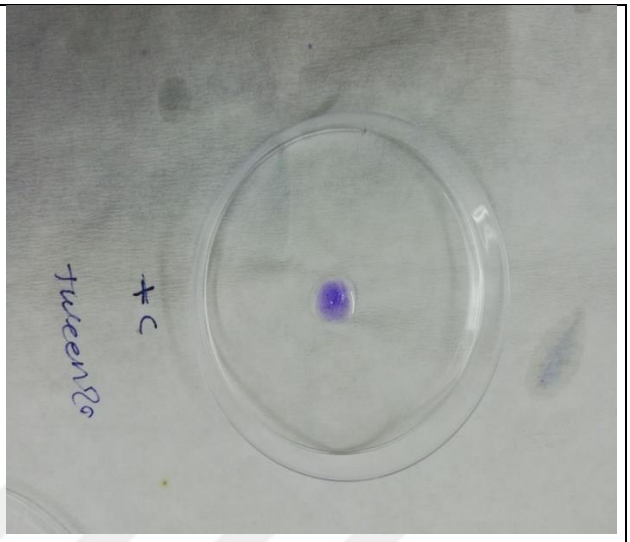
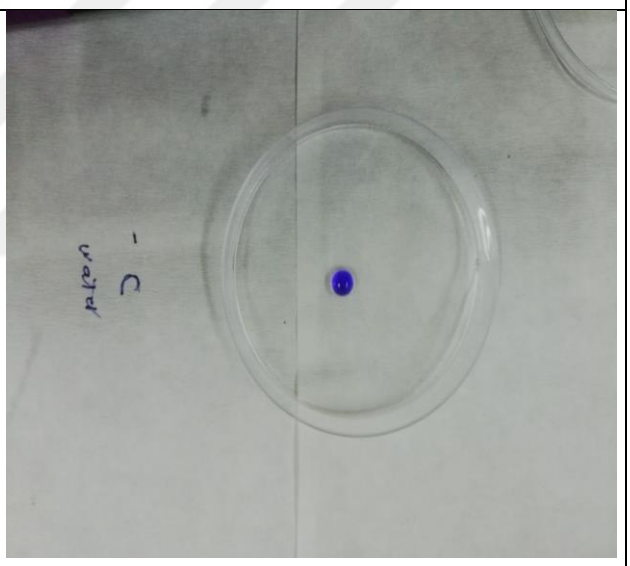
	1 min	0.55 cm	
B.halotolerans	0 min	0.6 cm	

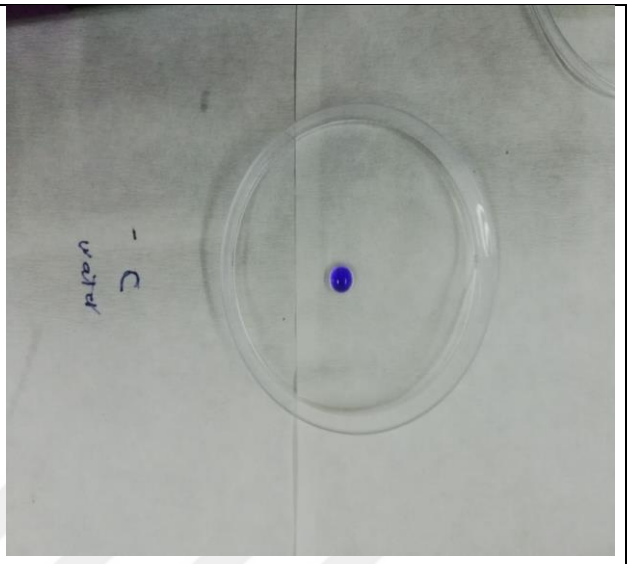
	1 min	0.7 cm	
M.arborescens	0 min	0.5 cm	

	1 min	0.6 cm	
P.veronii	0 min	0.6 cm	

	1 min	0.65 cm	
P.reinekei	0 min	0.6 cm	

	1 min	0.75 cm	
positive control (tween 80)	0 min	0.6 cm	

	1 min	0.9 cm	
negative control (distilled water)	0 min	0.5 cm	

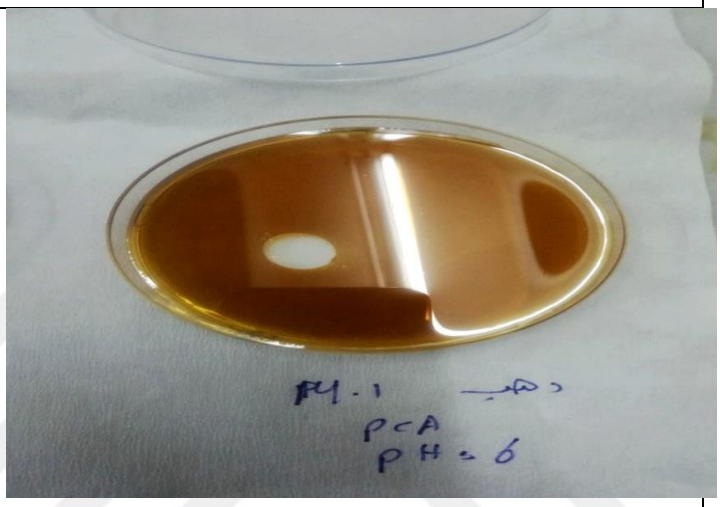
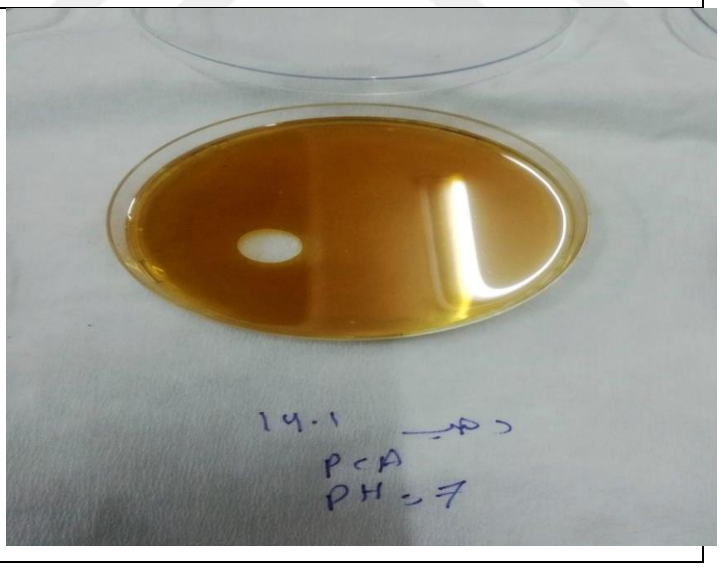
	1 min	0.5 cm	

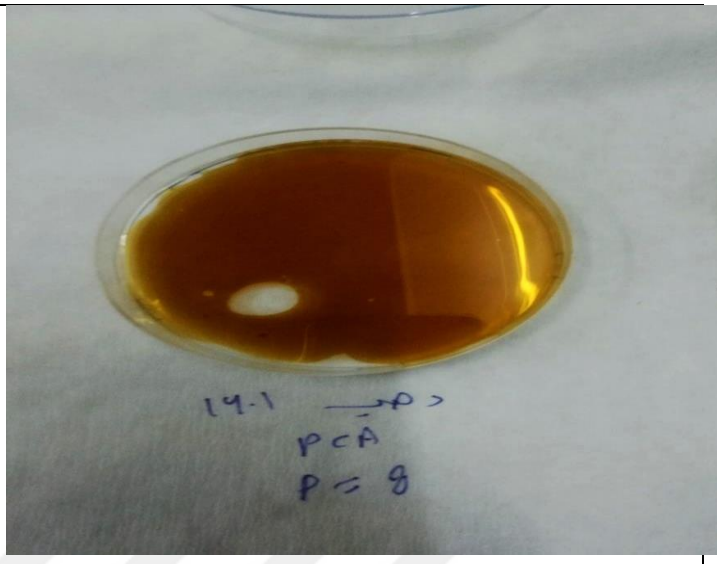
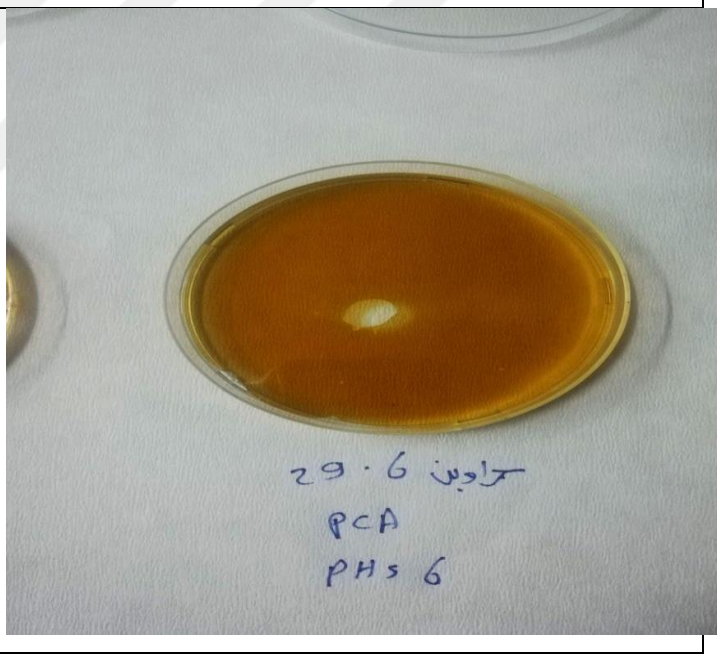
4.4. Optimization of Biosurfactant Produced by Bacteria

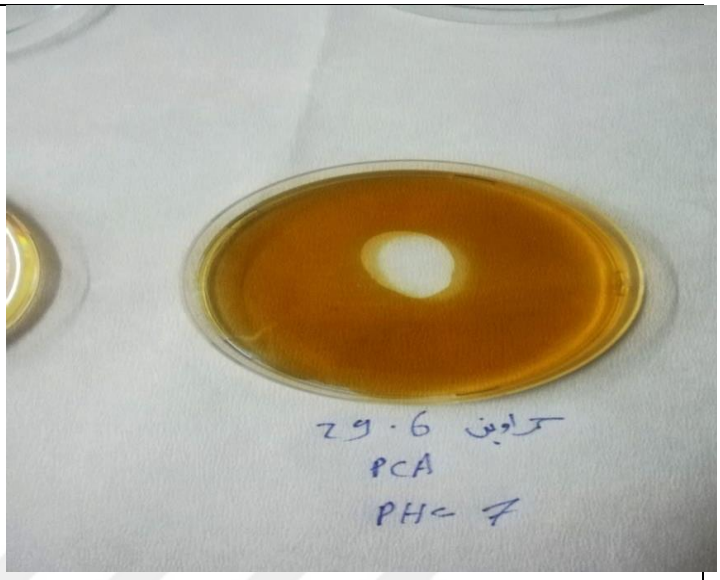
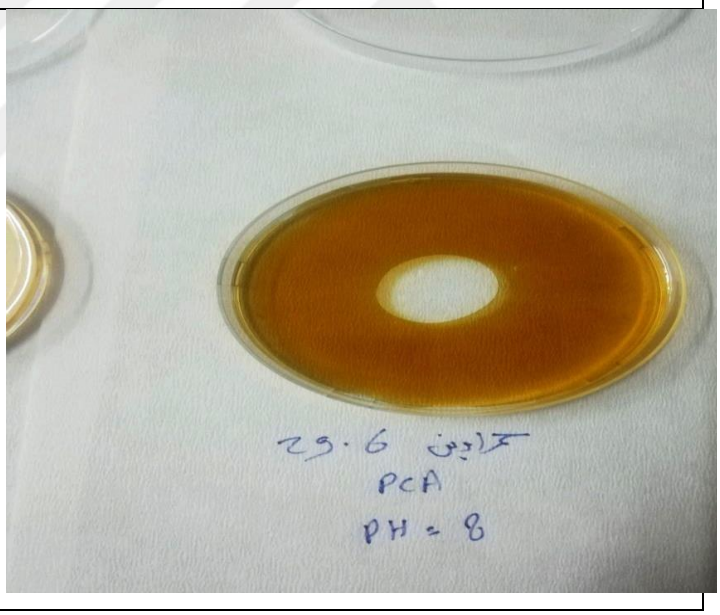
4.4.1. Effect of pH

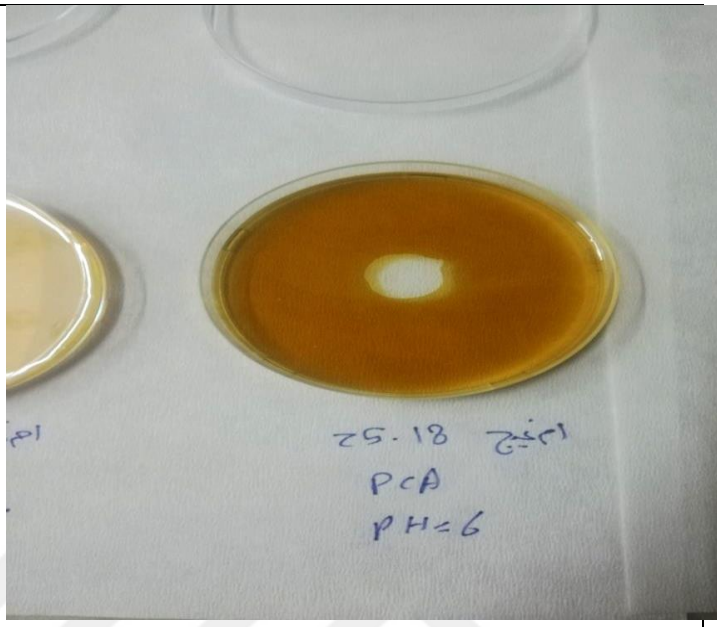
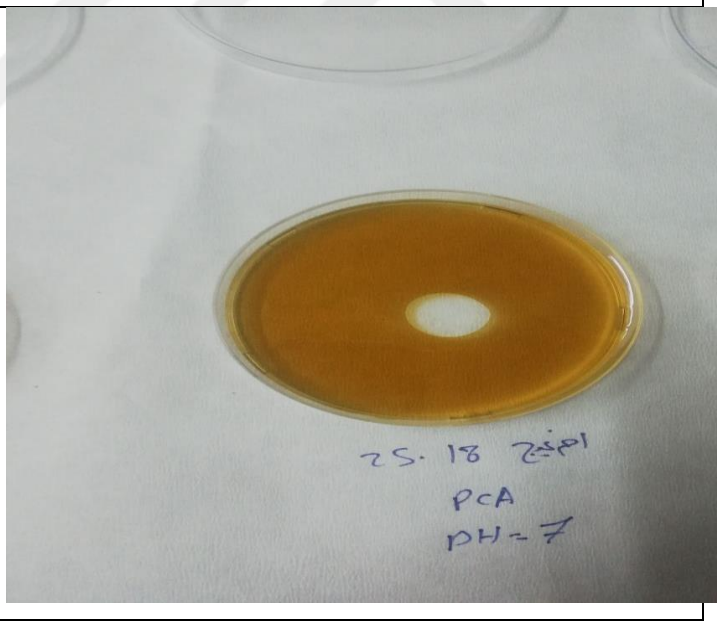
3 pH values were selected, 6.0, 7.0 and 8.0. Biosurfactant production Culture medium was prepared by adding 5% olive oil as the carbon source and pH was adjusted by pH meter using 6.0 M HCl and 0.1M NaOH solutions. After pH adjustment, the medium was sterilized, inoculated and incubated at 37°C for 3 days in orbital shaker at 150 rpm. Biosurfactant activity were determined by measuring the zone diameters with the oil spreading technique.

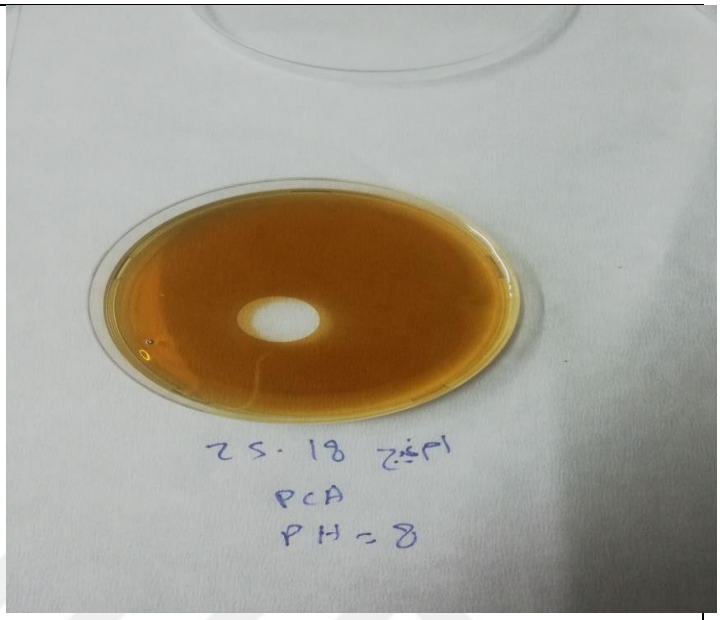
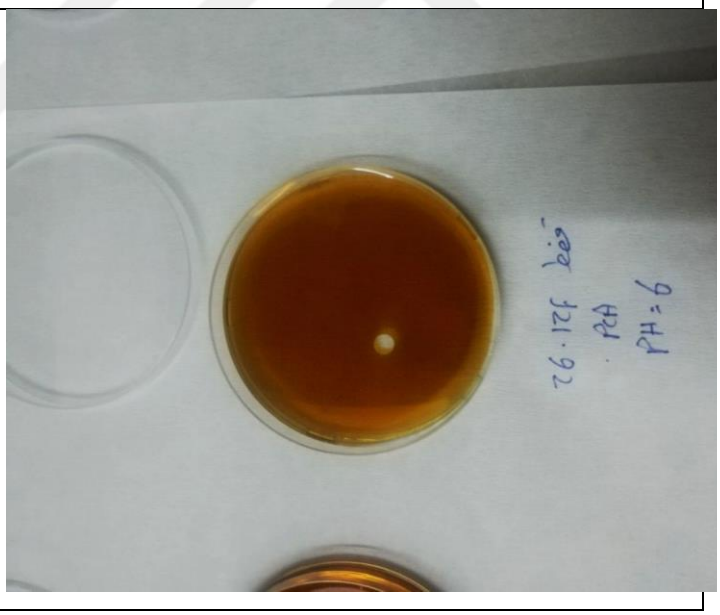
Table 4.22. Effect of pH on biosurfactant activity.

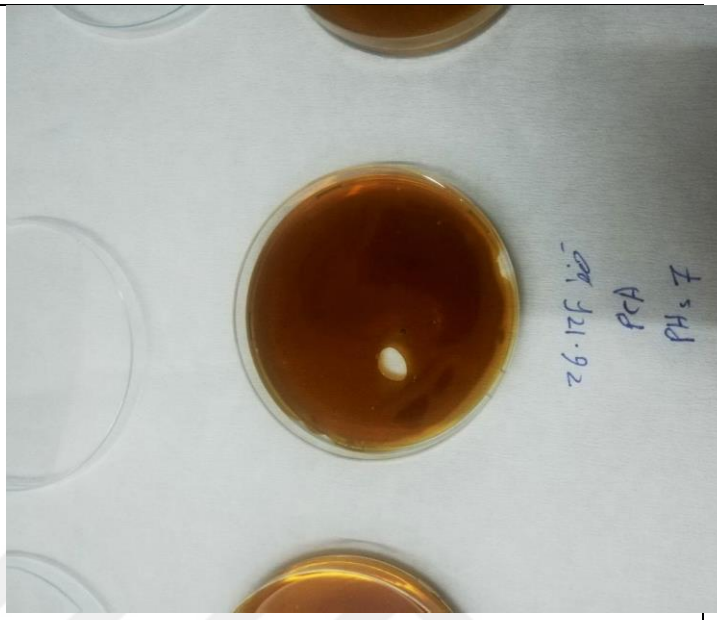
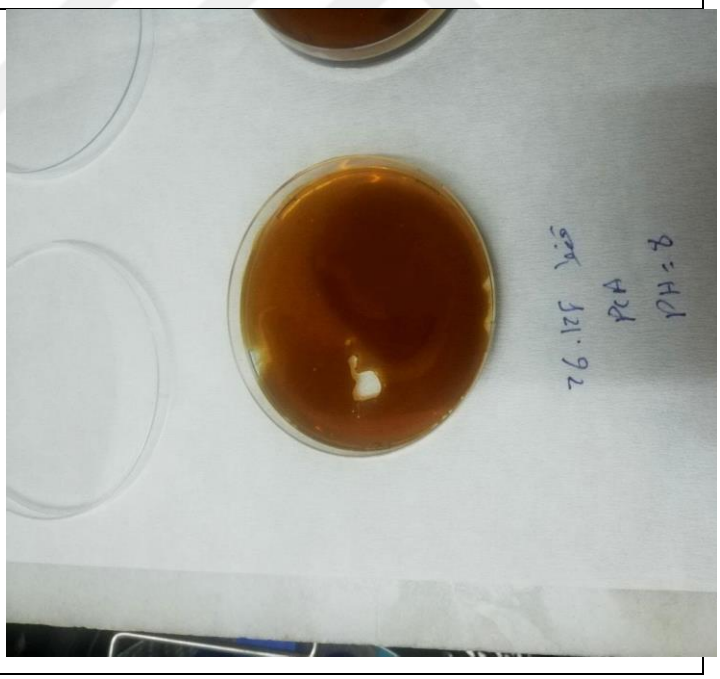
microorganism	pH / oil-free clearing zone diameter		Figure
B.velezensis	pH 6	1.65 cm	
	pH 7	1.5 cm	

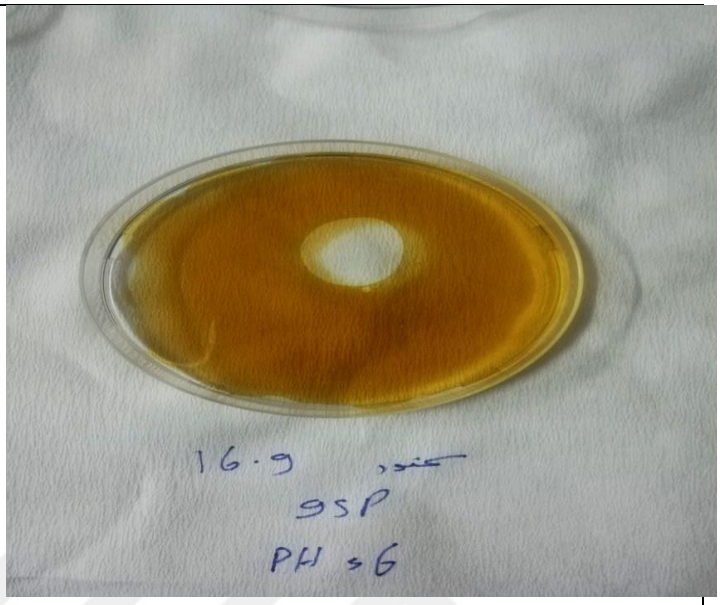
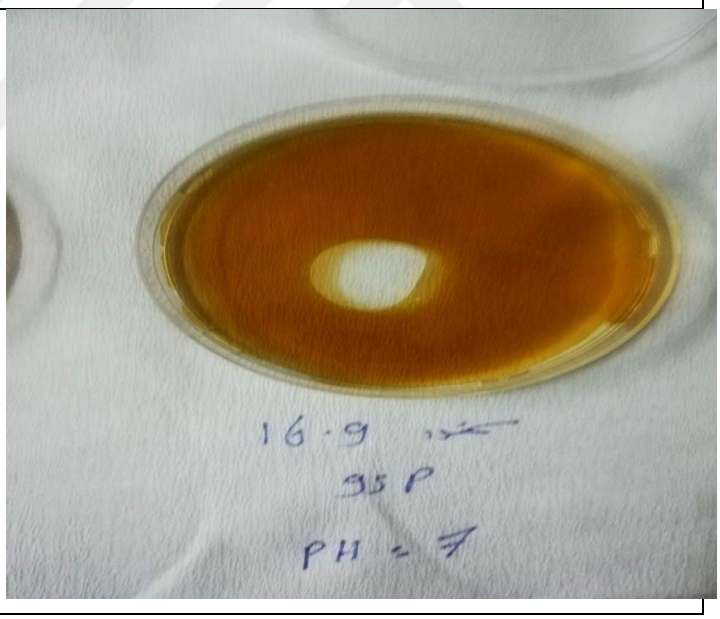
	pH 8	1.4 cm	
B.halotolerans	pH 6	0.9 cm	

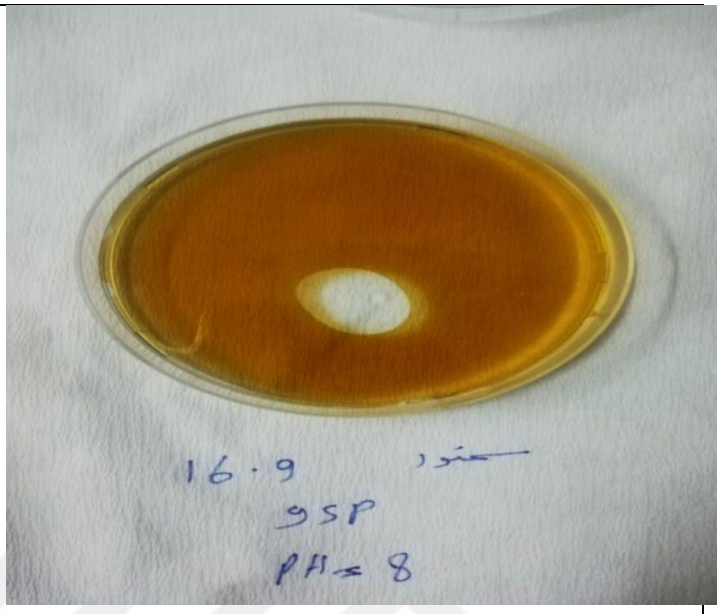
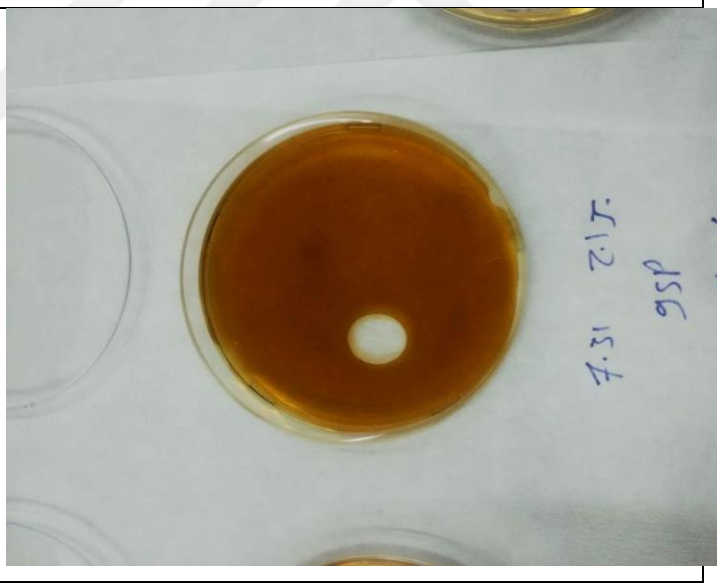
	pH 7	2.0 cm	
	pH 8	2.5 cm	

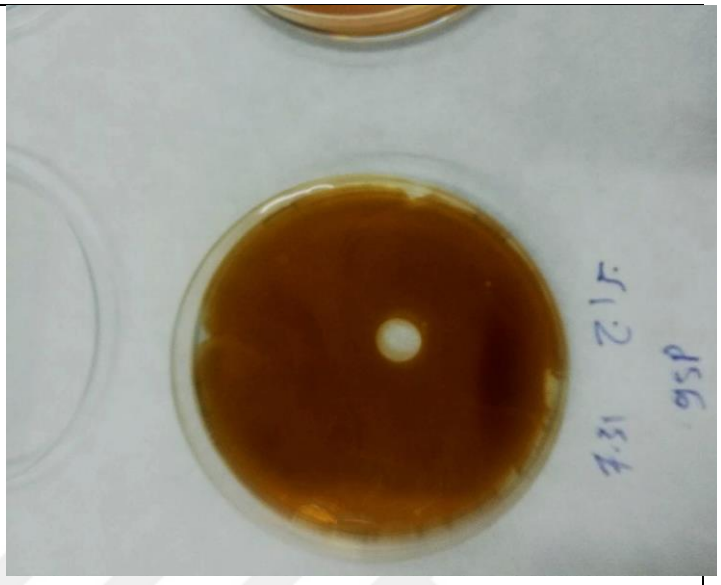
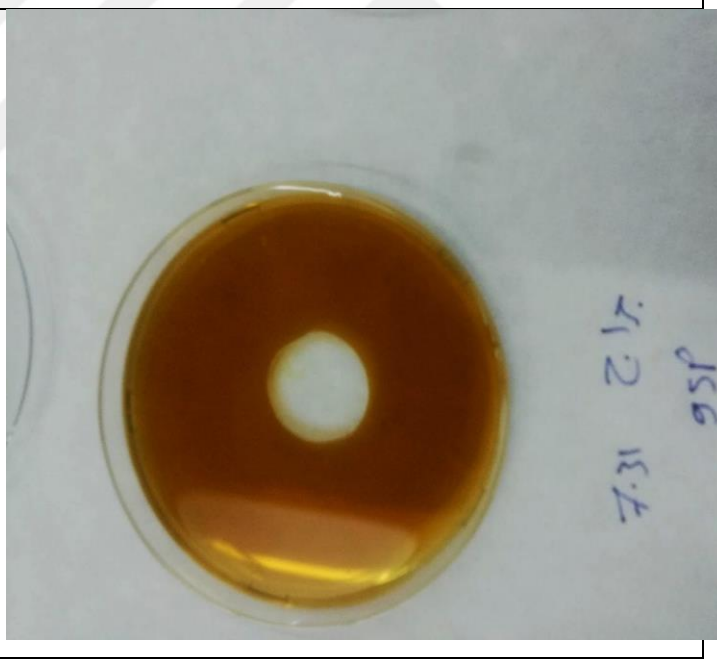
A.lwoffii	pH 6	1.7 cm	
	pH 7	2.0 cm	

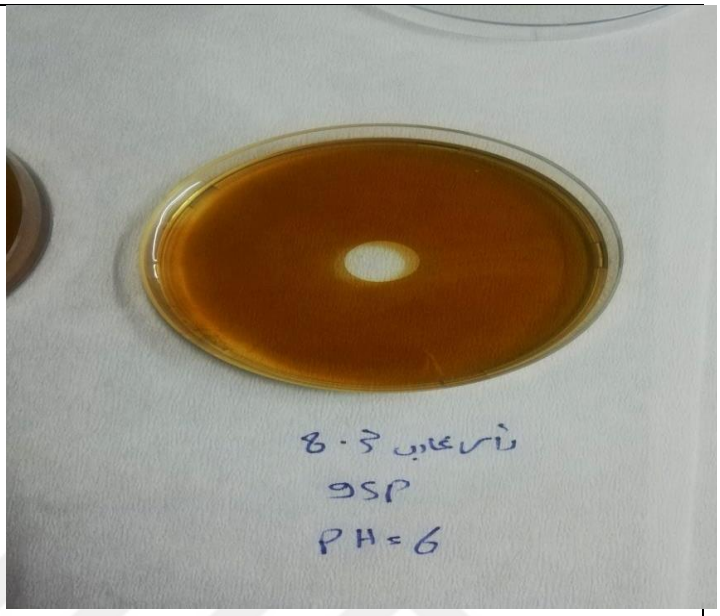
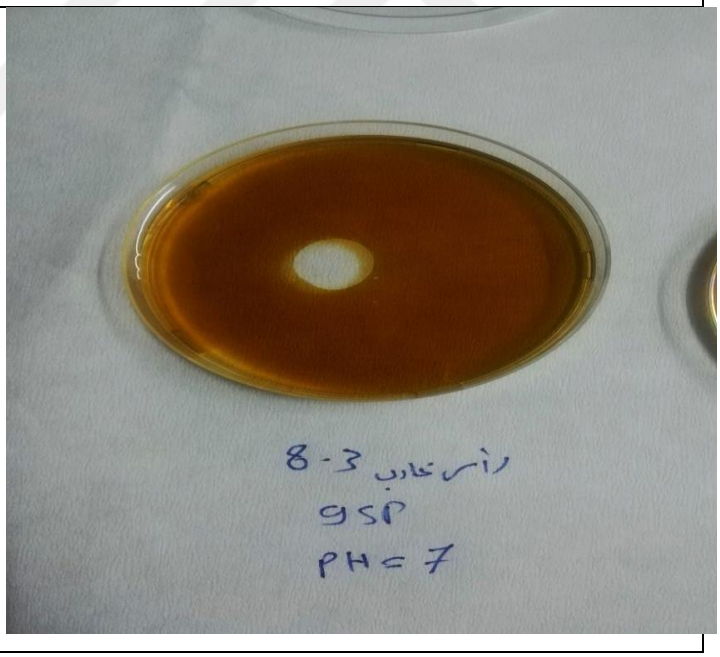
	pH 8	1.8 cm	
B.haynesii	pH 6	0.8 cm	

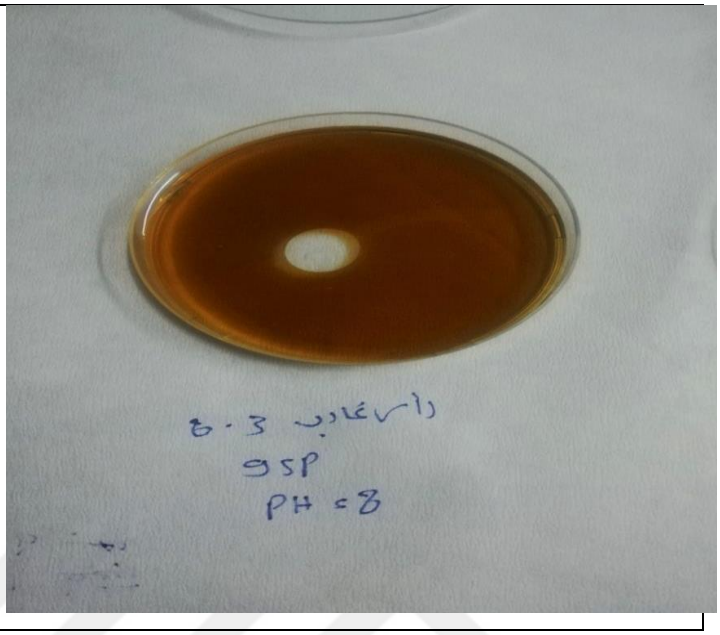
	pH 7	1.1 cm	
	pH 8	1.1 cm	

P.reinekei	pH 6	2.5 cm	
	pH 7	2.2 cm	

	pH 8	2.0 cm	
M.arboresce ns	pH 6	1.5 cm	

	pH 7	1.1 cm	
	pH 8	2.2 cm	

P.veronii	pH 6	1.5 cm	
	pH 7	1.8 cm	

	pH 8	1.6 5 cm	

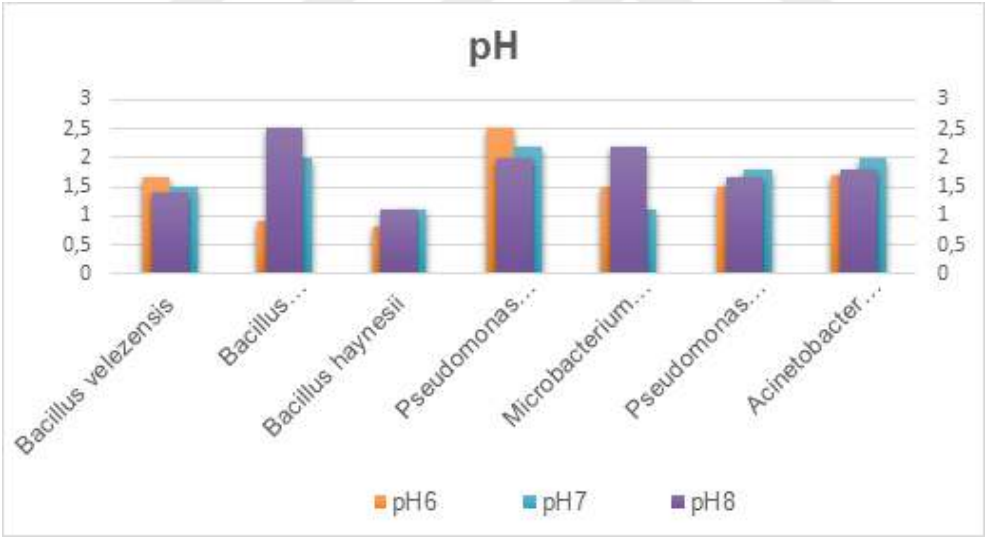
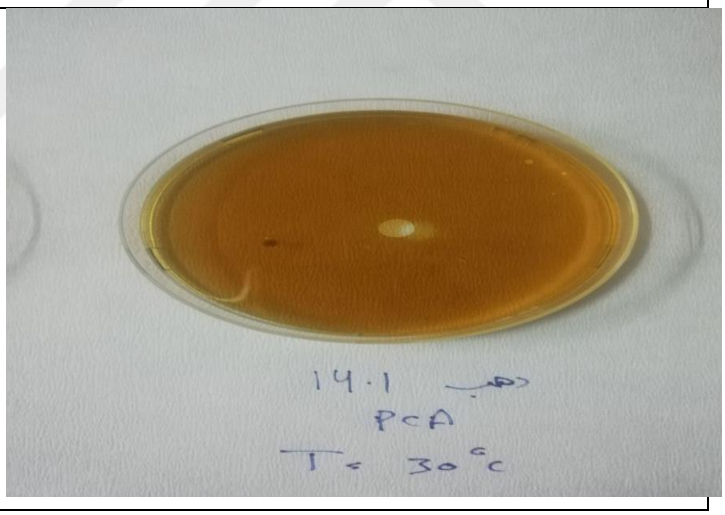


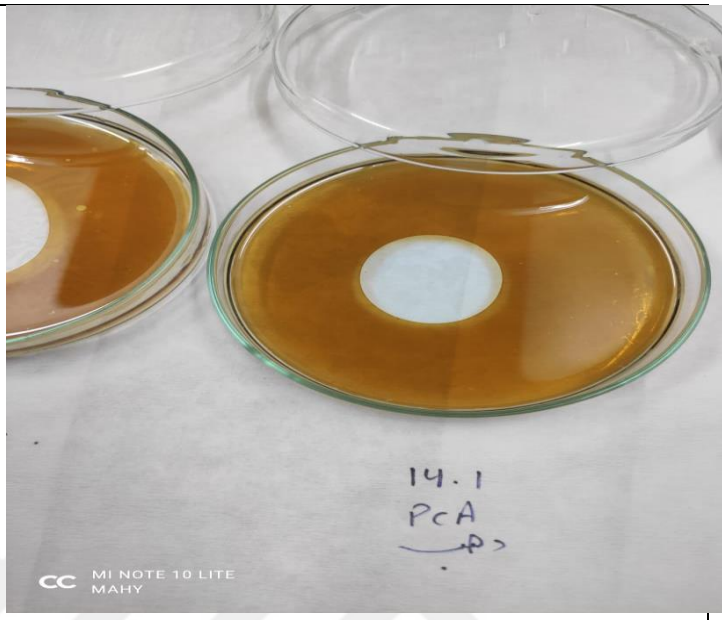
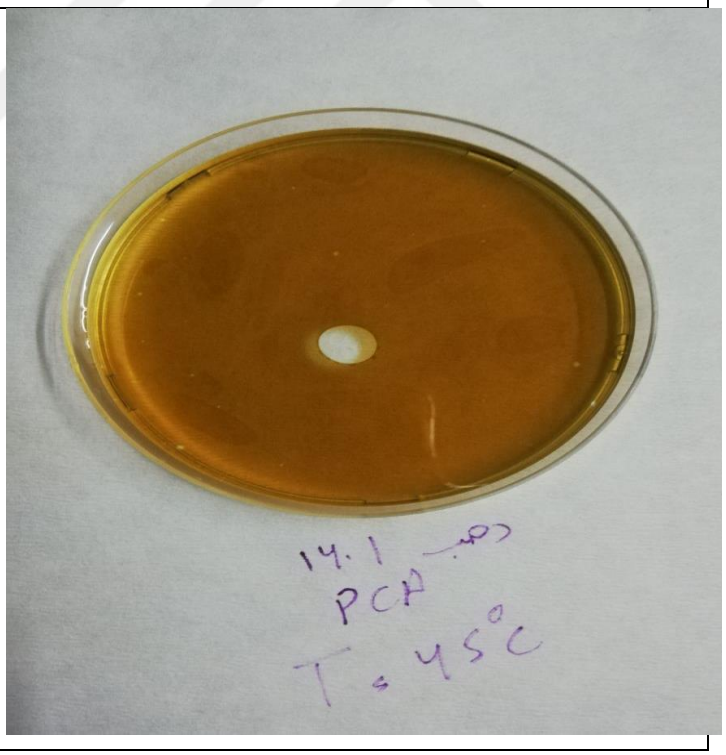
Figure 4.15. Effect of pH

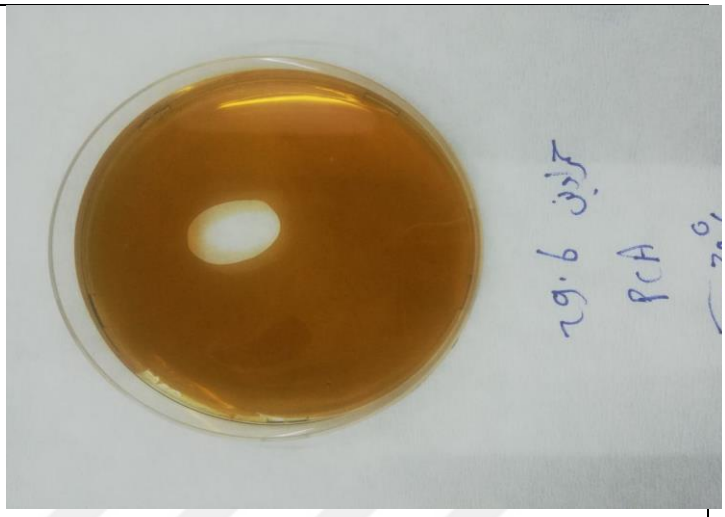
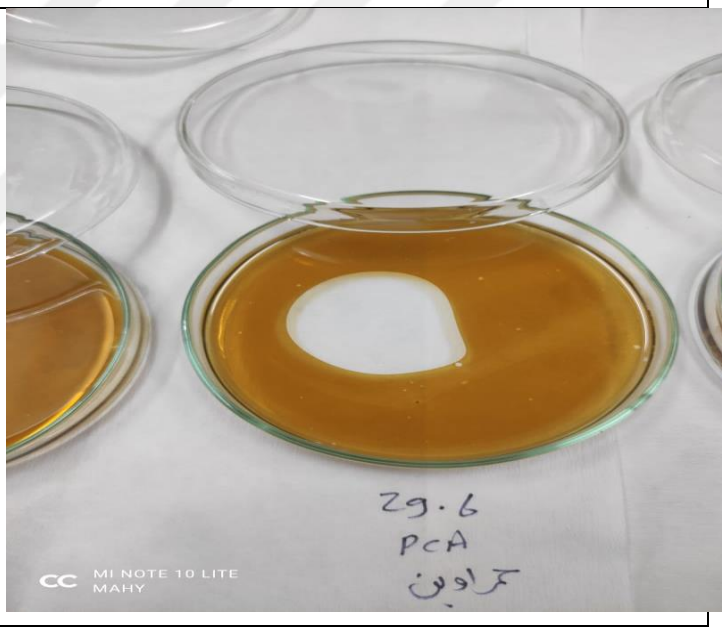
4.4.2. Effect of Temperature

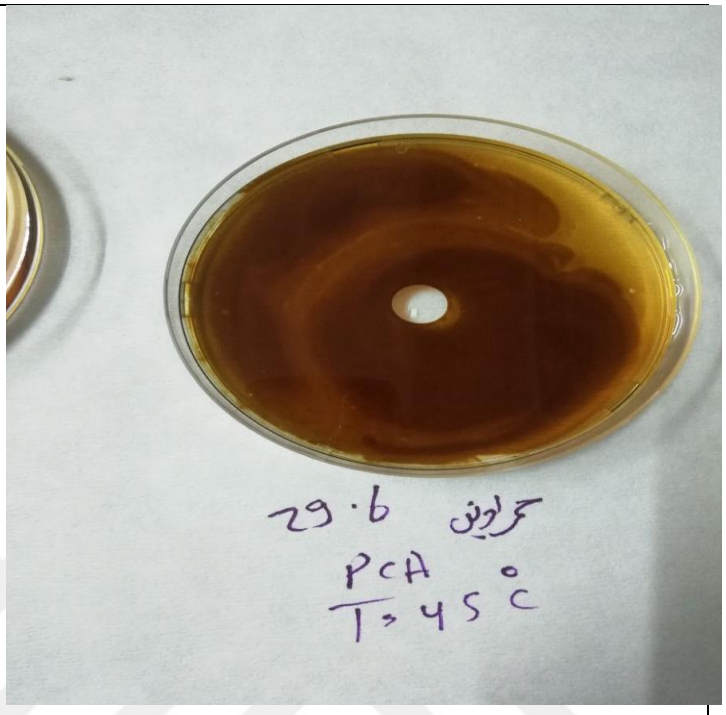
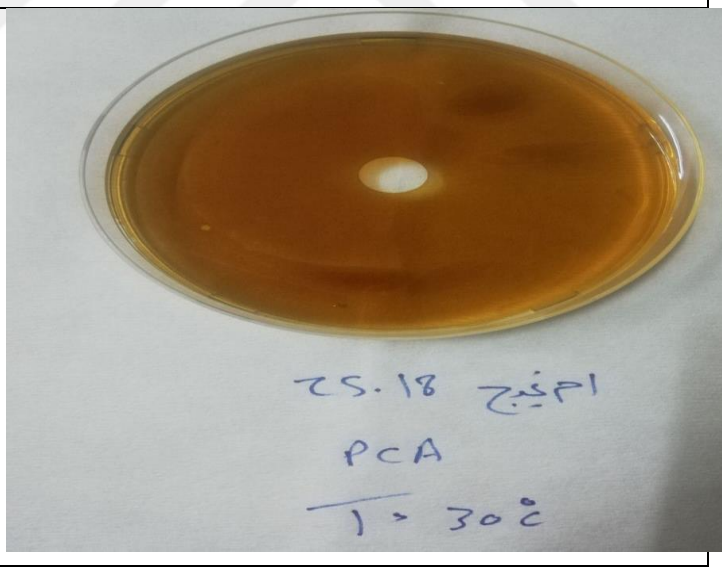
3 temperature values were selected, 30°C, 37°C and 45°C. Biosurfactant production Culture medium was prepared by adding 5% olive oil as the carbon source and pH was adjusted to 7.0 sterilized at 121°C for 15 min. Activated culture of biosurfactant producing bacteria was inoculated and incubated at different temperatures for 3 days in orbital shaker at 150 rpm. Biosurfactant activity were determined by measuring the zone diameters with the oil spreading technique.

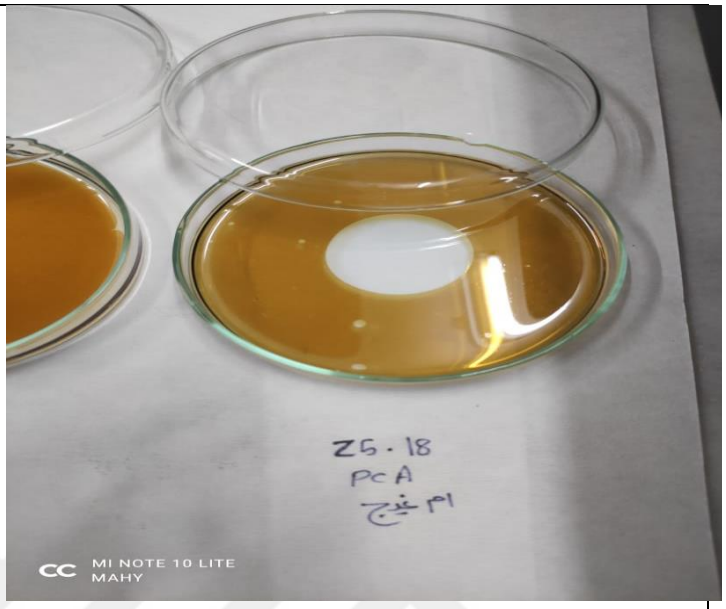
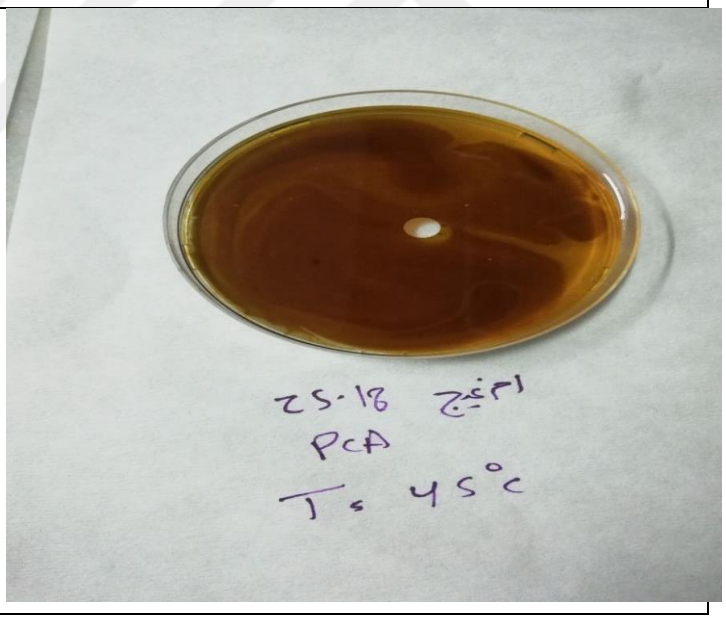
Table 4.23. Effect of Temperature on biosurfactant activity.

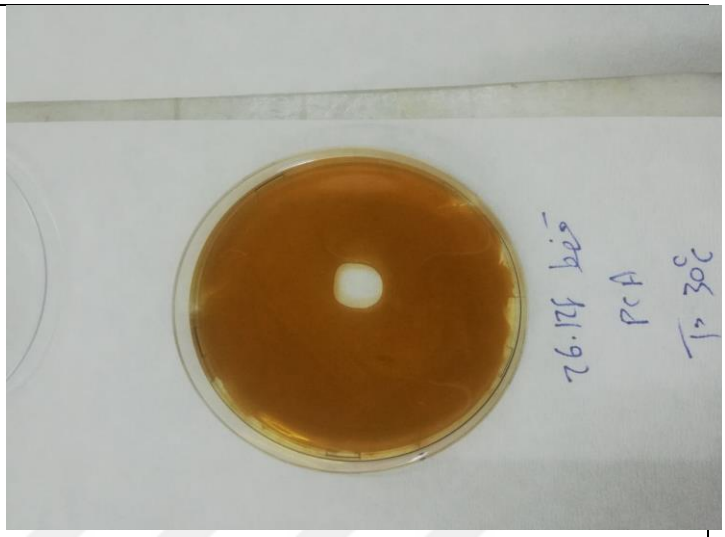
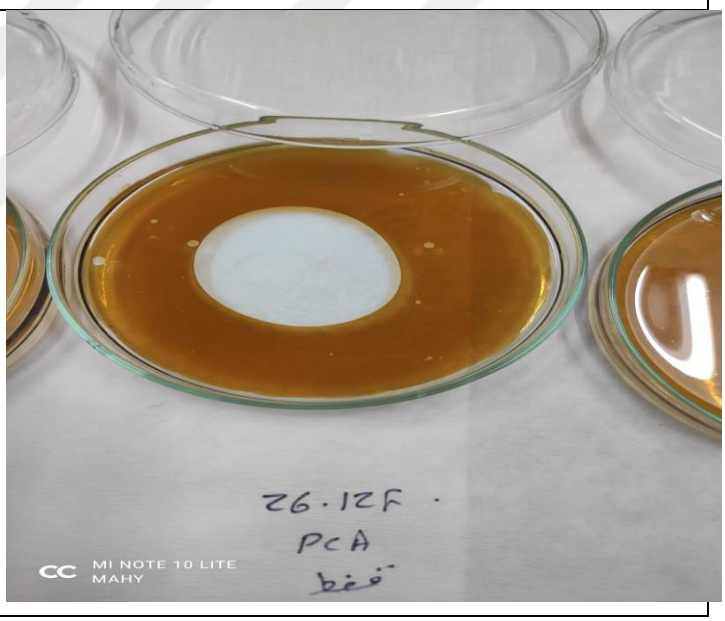
microorganism	Temperature / oil-free clearing zone diameter		Figure
B.velezensis	T 30°C	1.0 cm	

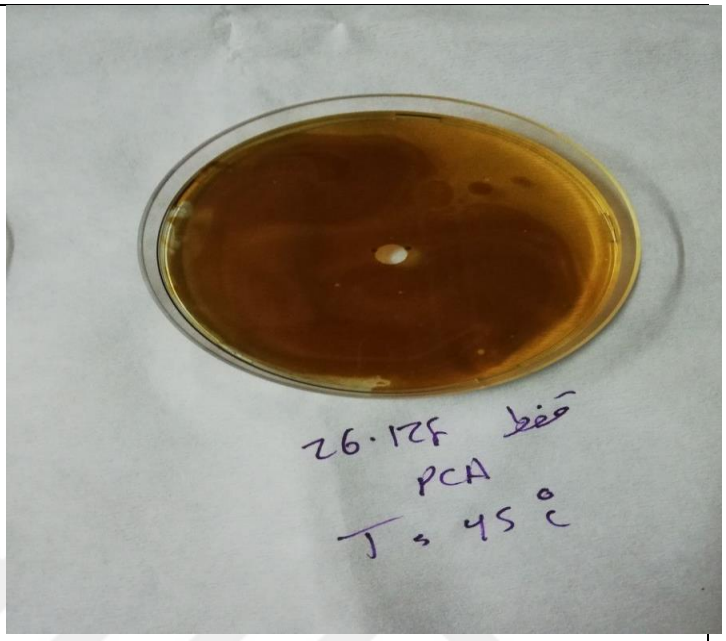
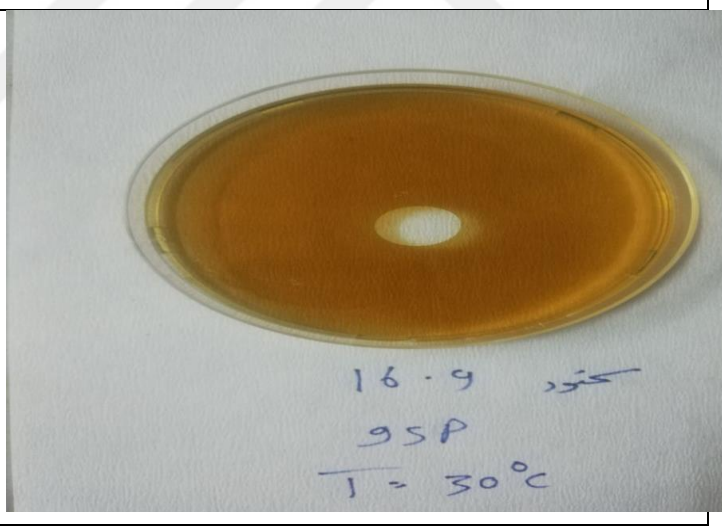
	T 37°C	4.0 cm	
	T 45°C	1.2 cm	

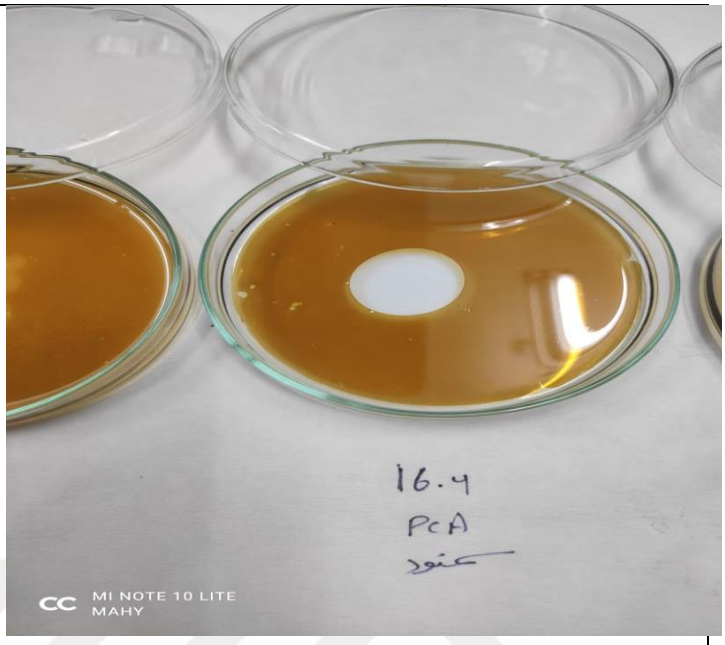
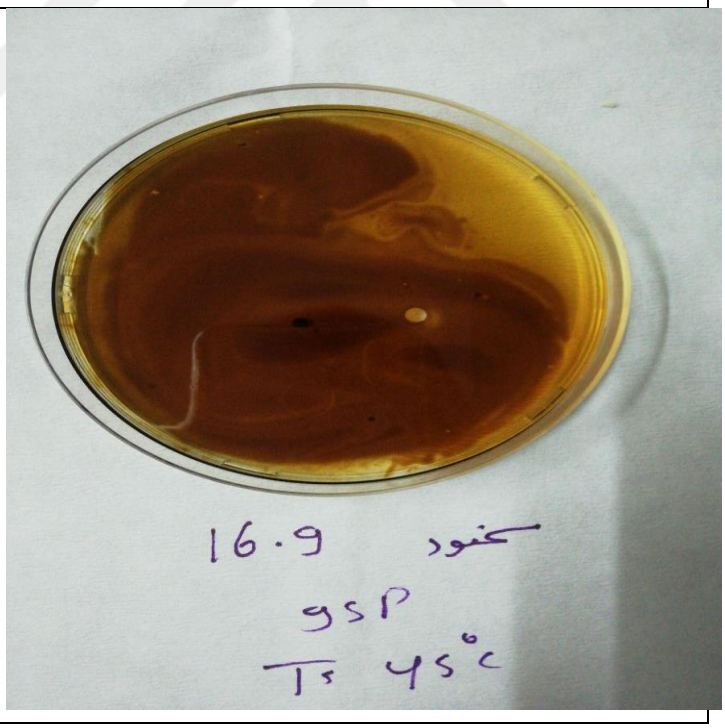
B.halotolerans	T 30°C	2.0 cm	
	T 37°C	5.0 cm	

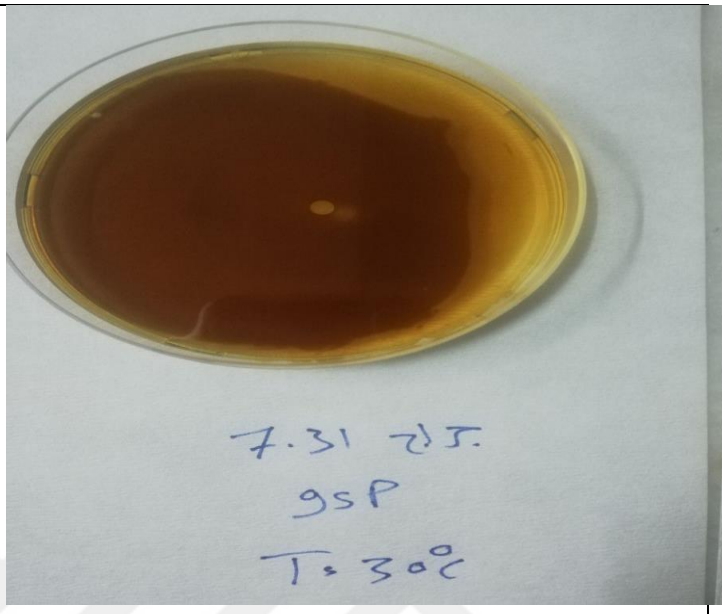
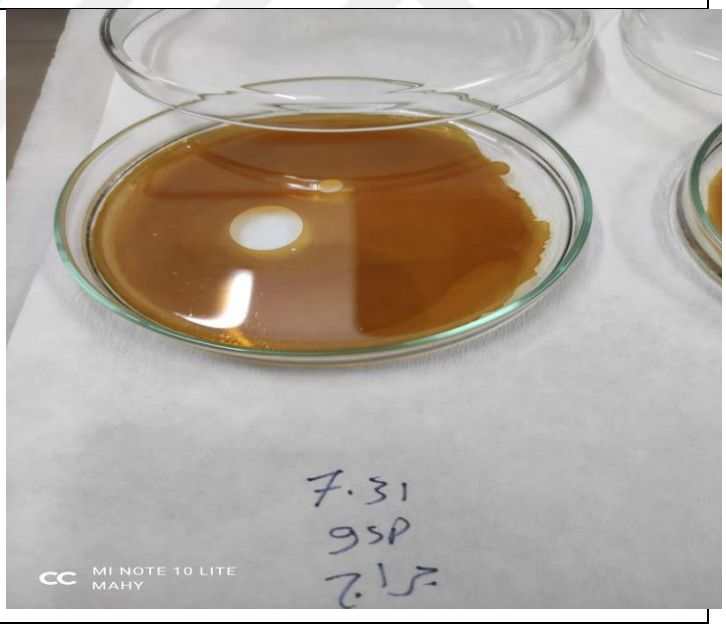
	T 45°C	1.25 cm	
A.lwoffii	T 30°C	1.15 cm	

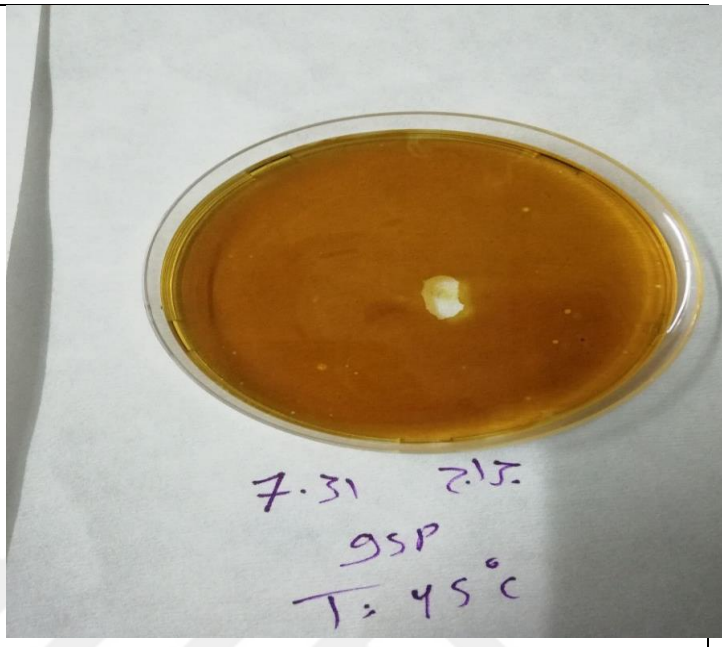
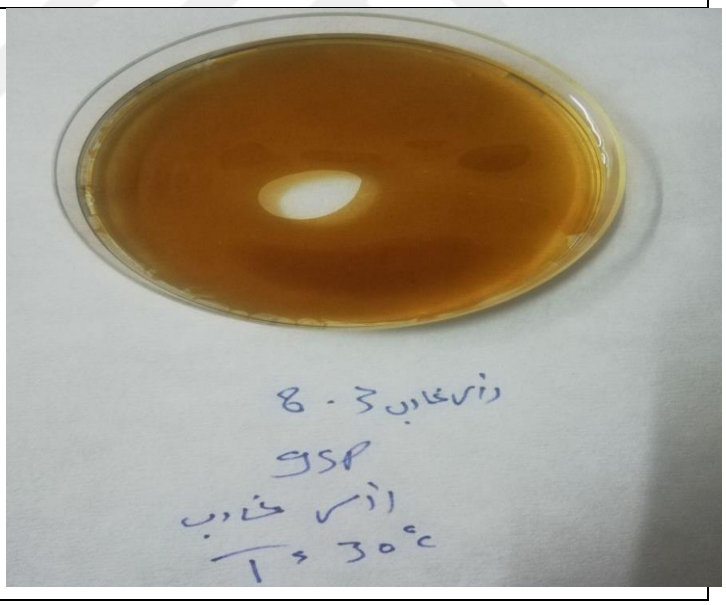
	T 37°C	2.7 cm	
	T 45°C	1.0 cm	

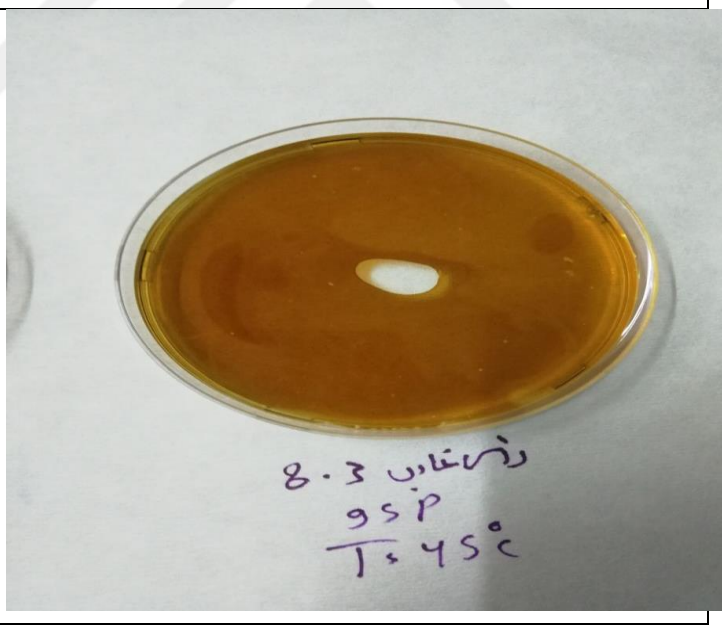
B.haynesii	T 30°C	1.4 cm	
	T 37°C	4.6 cm	

	T 45°C	0.8 cm	
P.reinekei	T 30°C	1.5 cm	

	T 37°C	4.0 cm	
	T 45°C	0.4 cm	

M.arboresce ns	T 30°C	1.0 cm	
	T 37°C	2.1 cm	

	T 45°C	1.2 cm	
P.veronii	T 30°C	1.7 cm	

	T 37° C	1.3 cm	
	T 45°C	1.0 cm	

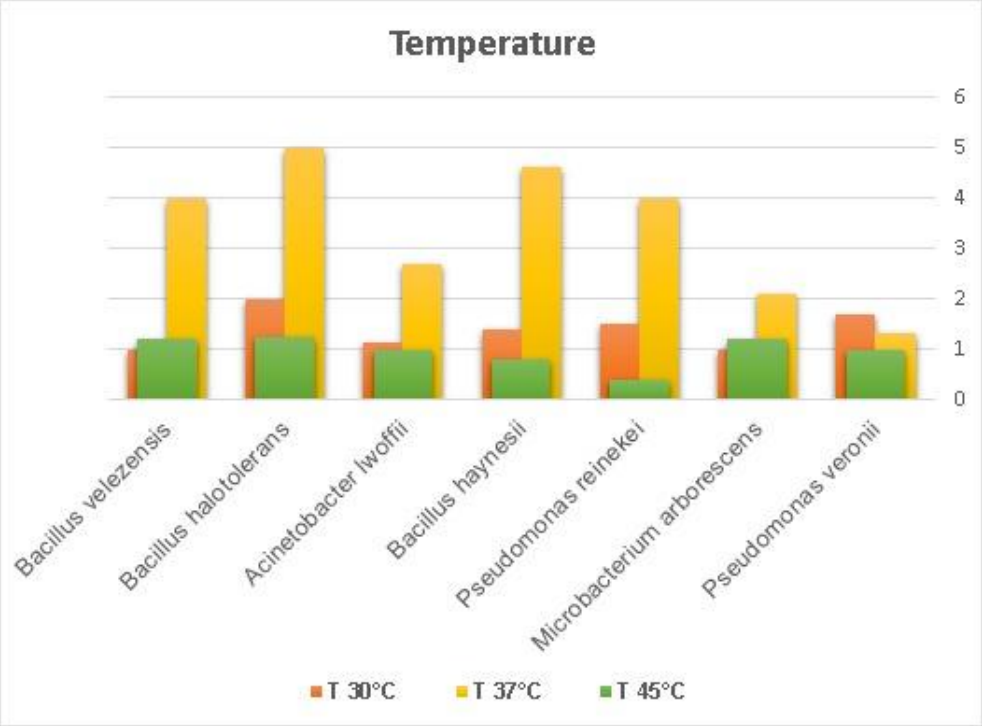
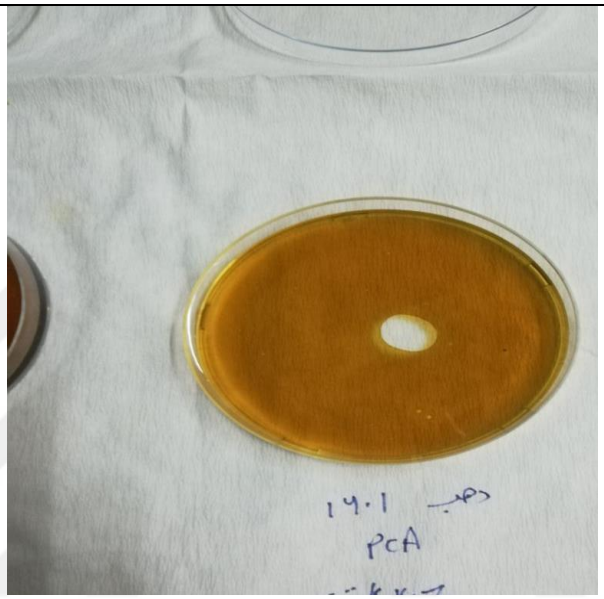
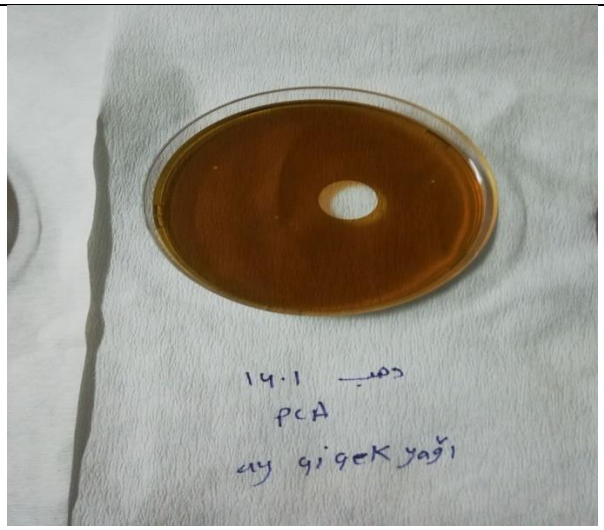


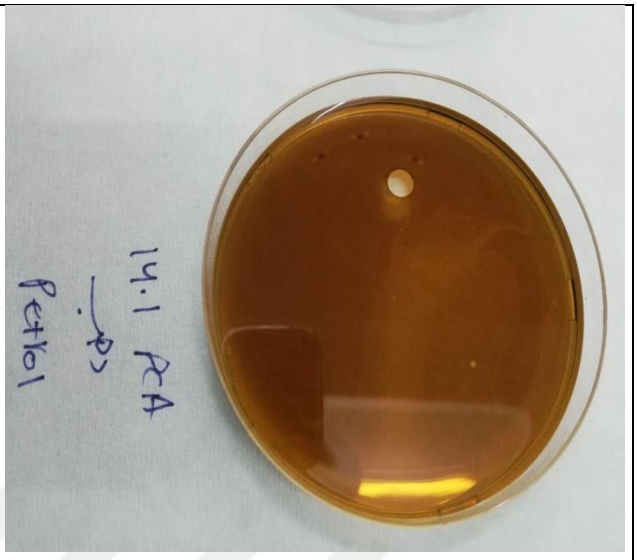
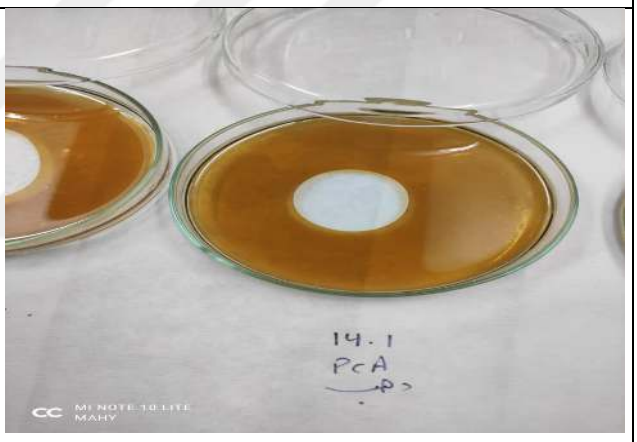
Figure 4.16. Effect of temperature

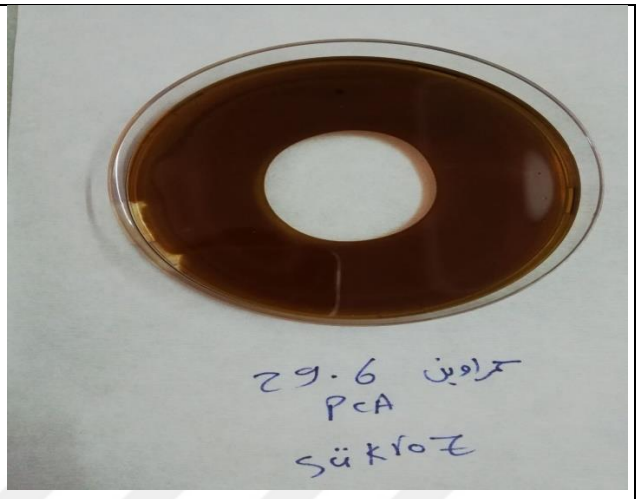
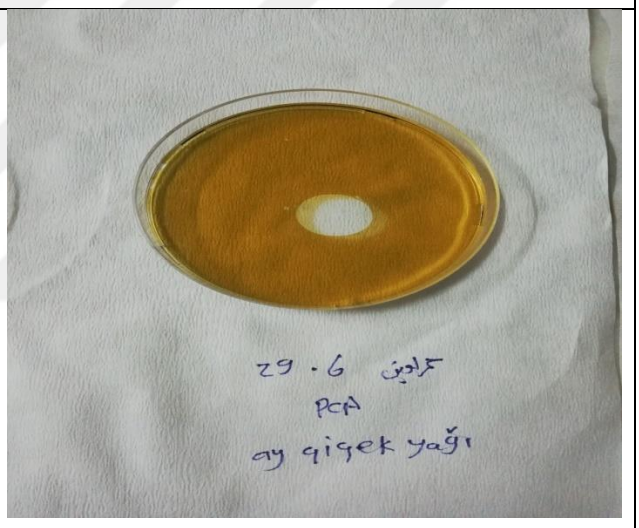
4.4.3. Effect of Carbon Source

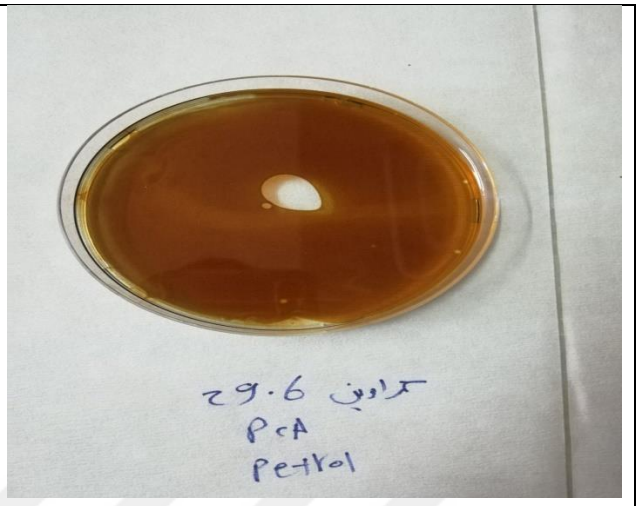
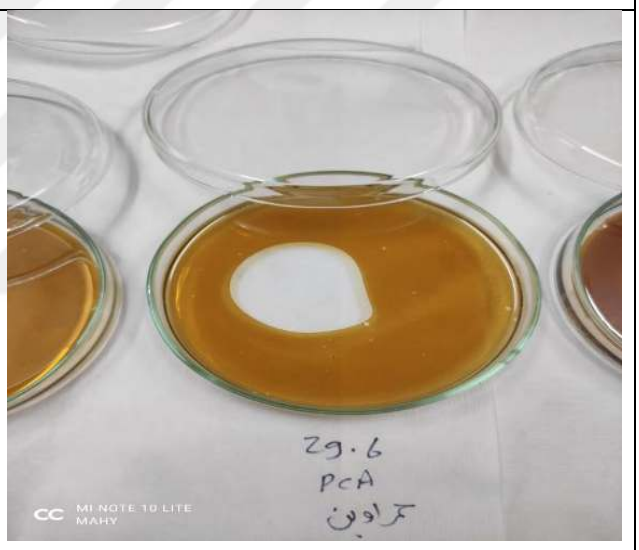
Four carbon sources were used, crude oil, olive oil, sunflower oil and sucrose. Biosurfactant production Culture medium was prepared by adding 5% of the carbon source and pH was adjusted to 7.0 by pH meter using 6.0 M HCl and 0.1M NaOH solutions. After pH adjustment, the medium was sterilized, inoculated and incubated at 37°C for 3 days in orbital shaker at 150 rpm. Biosurfactant activity were determined by measuring the zone diameters with the oil spreading technique.

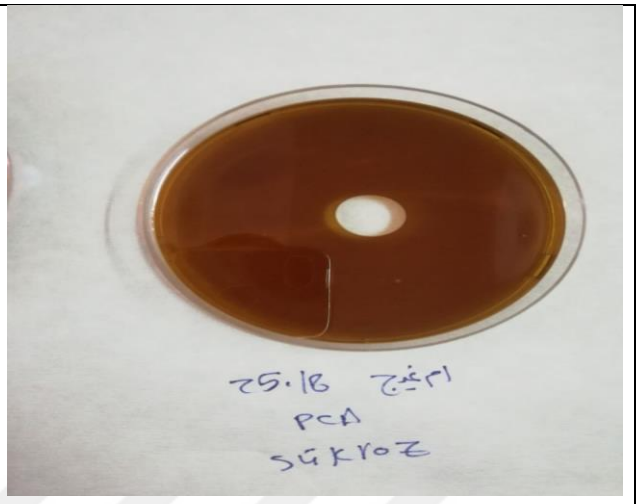
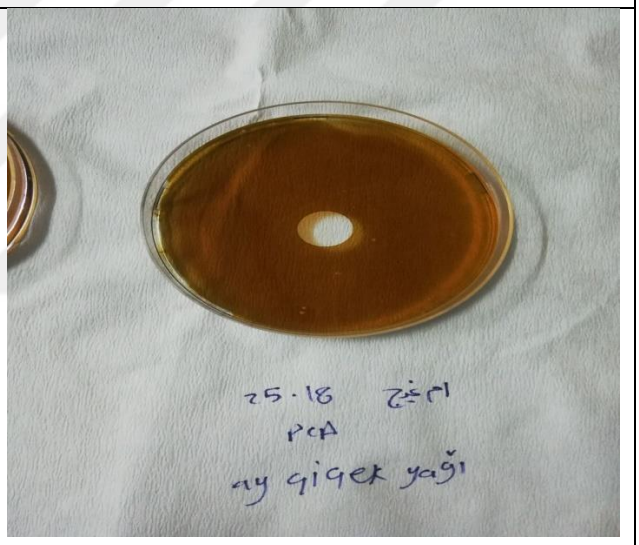
Table 4.24. Effect of Carbon source on biosurfactant activity.

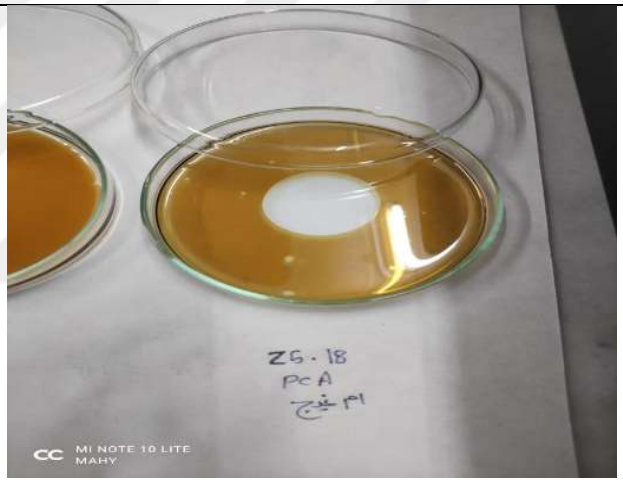
microorganism	Carbon source / oil-free clearing zone diameter		Figure
B.velezensis	sucrose	1.4 cm	
	sunflower oil	1.7 cm	

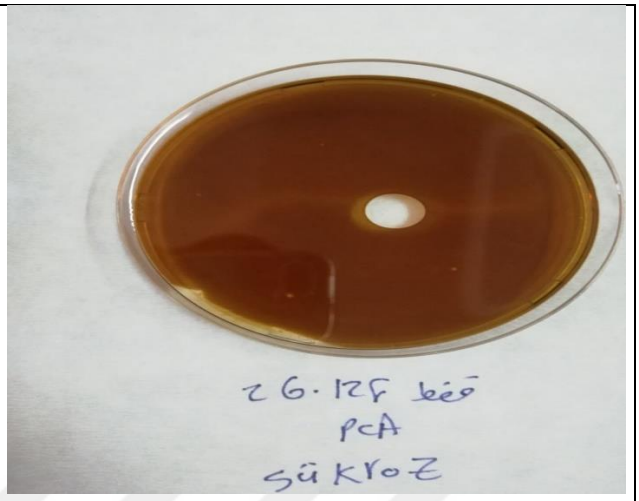
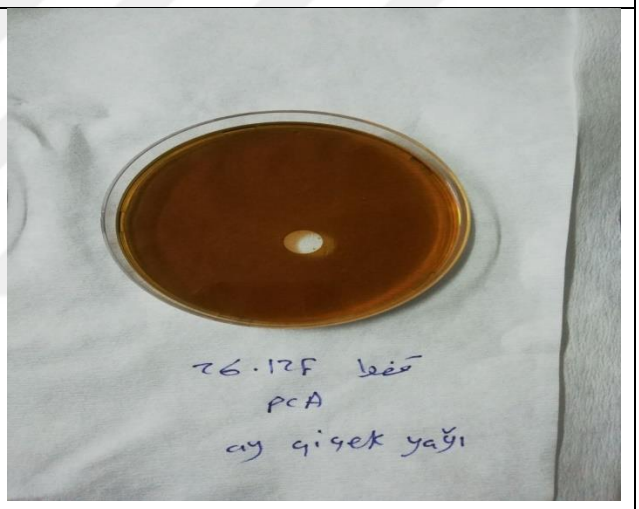
	crude oil	0.85 cm	
	olive oil	4.0 cm	

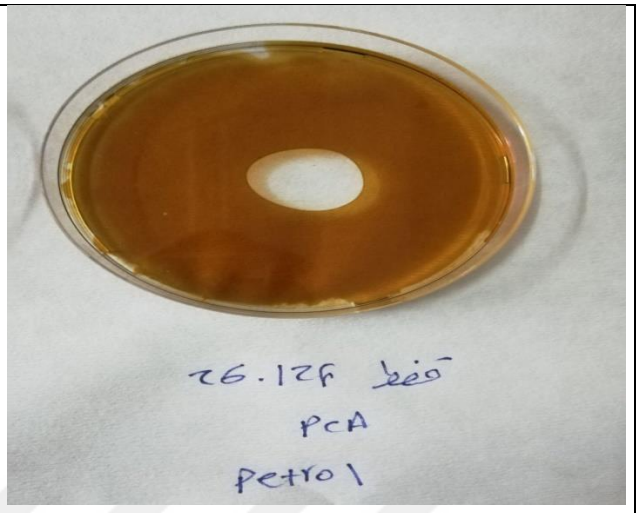
B.halotolerans	sucrose	3.8 cm	
	sunflower oil	2.1 cm	

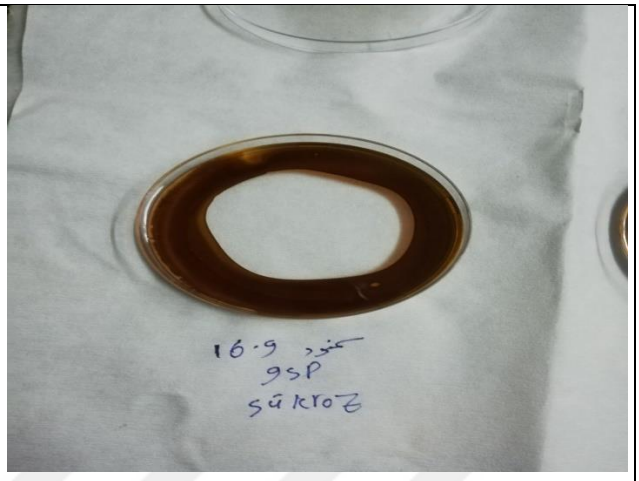
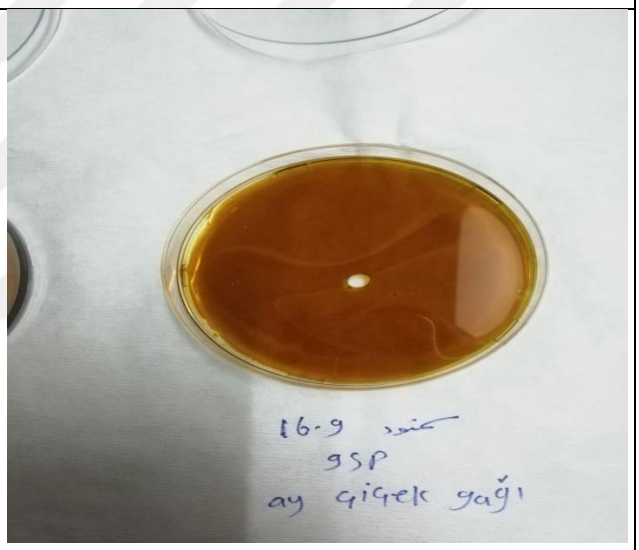
	crude oil	1.35 cm	
	olive oil	5.0 cm	

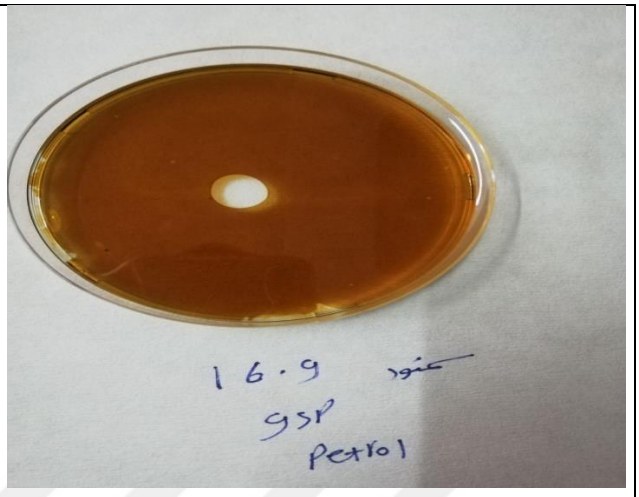
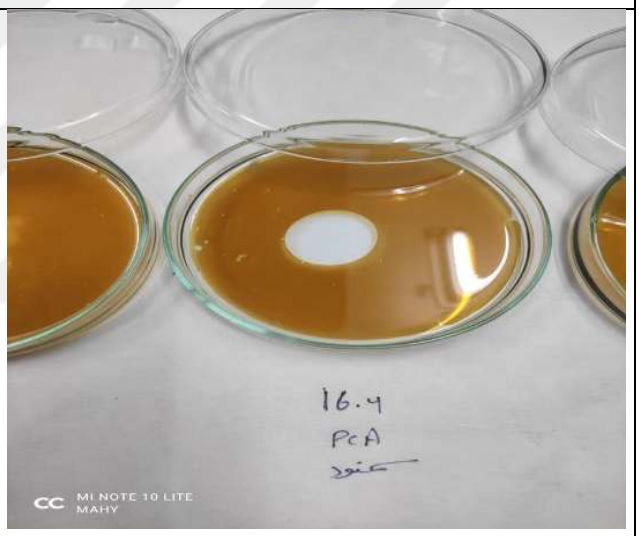
A.lwoffii	sucrose	1.7 cm	
	Sunflower oil	1.5 cm	

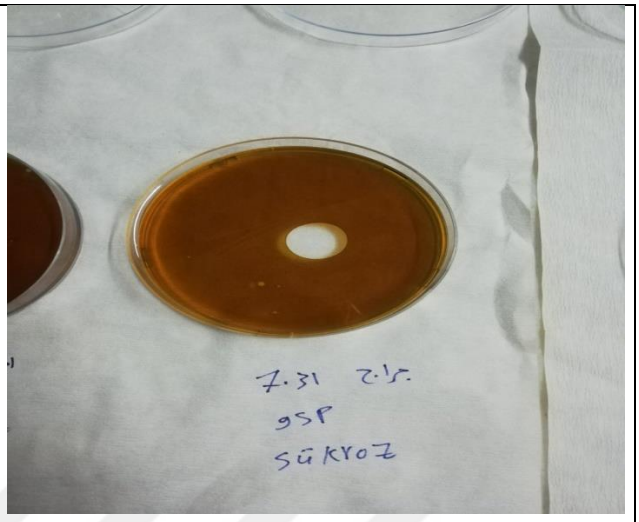
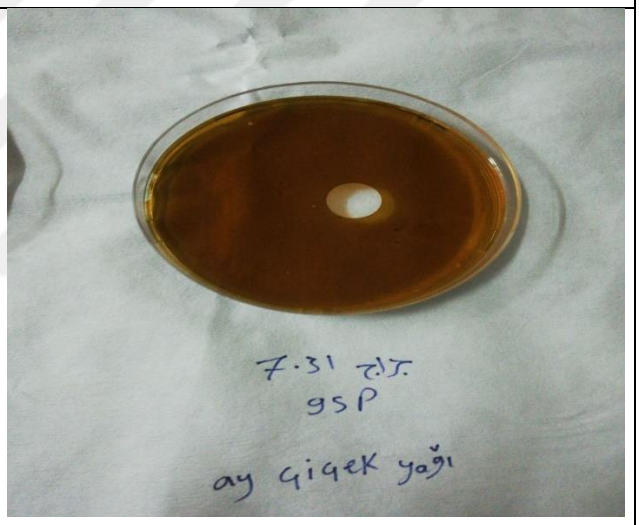
	crude oil	1.1 cm	
	olive oil	2.7 cm	

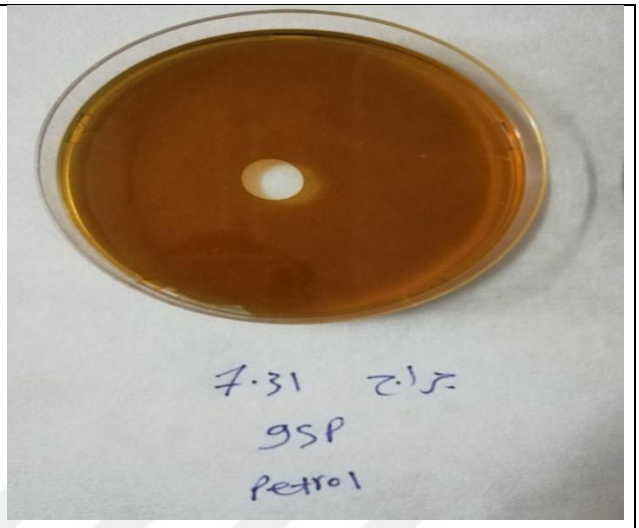
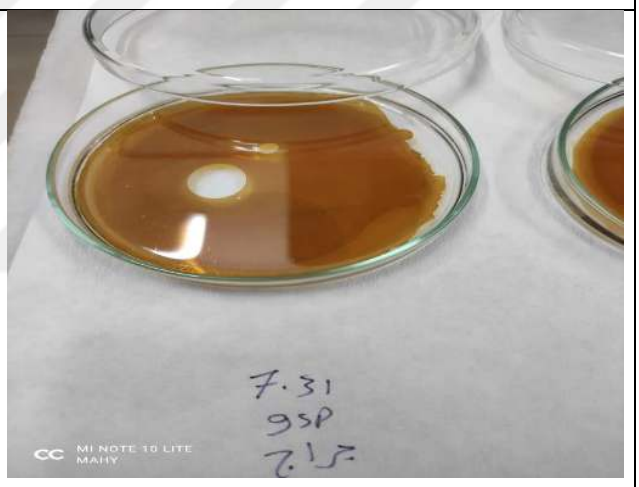
B.haynesii	sucrose	1.35 cm	
	Sunflower oil	1.1 cm	

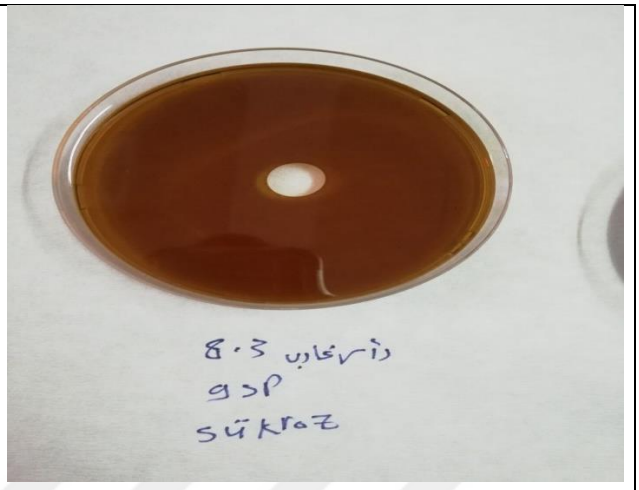
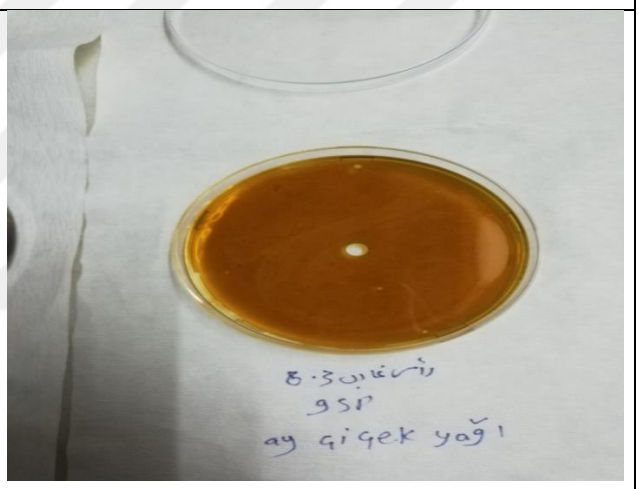
	crude oil	2.2 cm	
	olive oil	4.6 cm	

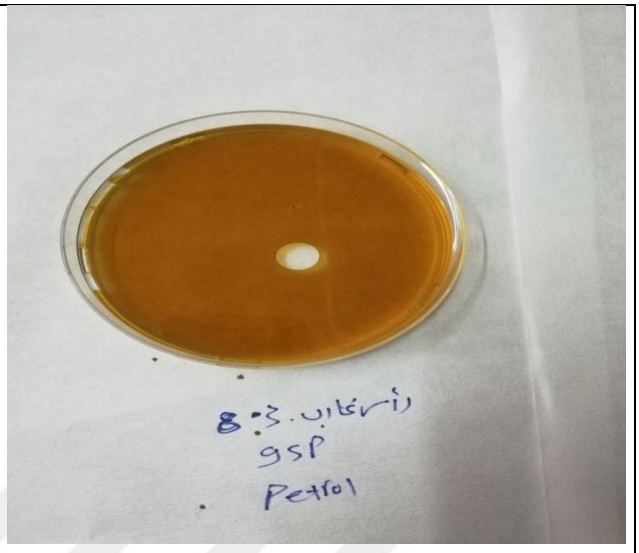
P.reinekei	sucrose	5.7 cm	
	sunflower oil	0.6 cm	

	crude oil	1.25 cm	
	olive oil	4.0 cm	

M.arborescens	sucrose	1.8 cm	
	sunflower oil	1.6 cm	

	crude oil	1.35 cm	
	olive oil	2.1 cm	

P.veronii	sucrose	1.4 cm	
	sunflower oil	0.7 cm	

	crude oil	1.15 cm	
	olive oil	1.3 cm	

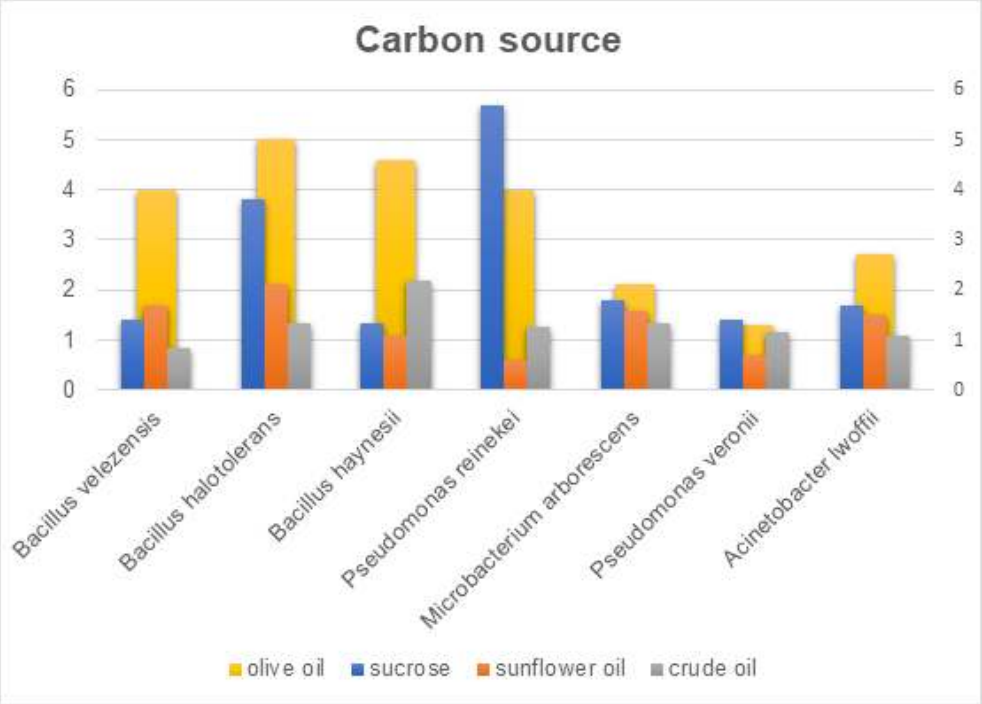


Figure 4.17. Effect of carbon source

4.5. Determination of Antibacterial and Antifungal Activity of Biosurfactant

Crude biosurfactant was used to assay for anti-microbial activity using well diffusion method. The crude biosurfactant was tested against *S.aureus ATCC 29213*. two kinds of *E.coli*, *Salmonella spp*., *Klebsiella spp*. and two kinds of *S.aureus* which were isolated from balcali hospital and *S.aureus* which was isolated from the nature for the antimicrobial (antibacterial) activity and *C.albicans* for the antimicrobial (antifungal) activity. For this purpose, stock cultures (100 µL) were inoculated into 5 mL Mueller-Hinton broth and incubated overnight in a shaking incubator at 150 rpm at 30°C. 0.1 mL of the *E.coli 1*, *E.coli 2*, *S.aureus 1* (isolated from hospital), *S.aureus 2* (isolated from nature), *S.aureus ATCC 29213*, *Salmonella spp*., *Klebsiella spp* suspensions were taken and cultivated in Mueller Hinton agar, for *C.albicans* stock cultures (100 µL) were inoculated into 5 mL malt extract broth and incubated overnight in a shaking incubator at 150 rpm at 30°C the

0.1 mL of the *C.albicans* was cultivated in Potato Dextrose Agar ,Then sterile empty antibiotic discs were placed in the cultivated agar media (Mueller Hinton agar and Potato Dextrose Agar) after absorbing the pure biosurfactant solution at 25, 50 and 100 µL concentrations and incubated at 30°C for 24-48 hours . At the end of incubation, the zone diameter around the discs was measured. Ampicillin and penicillin *G* were used as a positive control in the antibacterial assay and norfloxacin as a positive control in the antifungal assay (Fernandes et all. 2007).

Table 4.25. Antibacterial activity the biosurfactant produced by *B.velezensis*

microorganism	pathogen	Biosurfactant Concentration		
		25 µL	50 µL	100µL
B.velezensis	*E.coli 2*	-	0.7 cm	1.0 cm
	S.aureus(Clinical isolate) 1	-	1.1 cm	1.2 cm
	S.aureus (nature)	-	1.0 cm	1.2 cm
	S.aureus(Clinical isolate) 2	0.75 cm	1.2 cm	1.35 cm
	Salmonella spp.	-	0.8 cm	-
	E.coli 1	0.6 cm	0.75 cm	1.0 cm
	Klebsiella spp.	0.65 cm	0.7 cm	0.7 cm
	*S.aureus*ATCC 29213	-	-	-

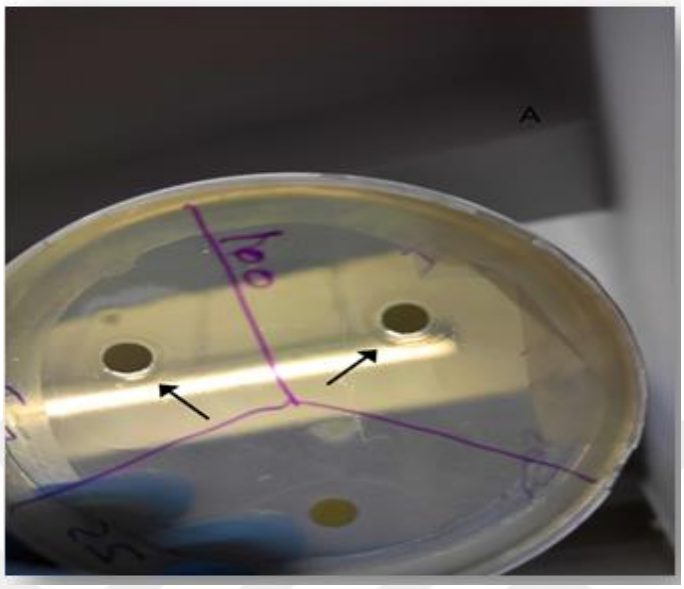


Figure 4.18. The biosurfactant produced by *B.velezensis* against *E.coli 2*

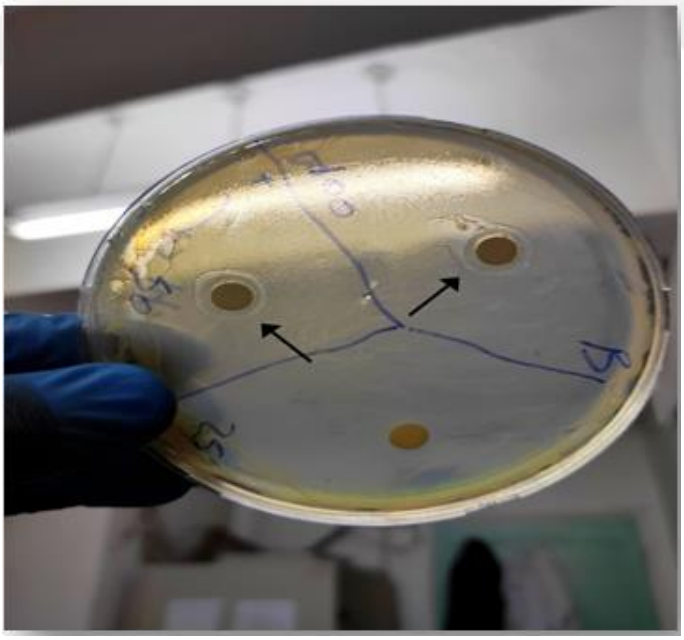


Figure 4.19. The biosurfactant produced by *B.velezensis* against *S.aureus* (Clinical isolate) 1

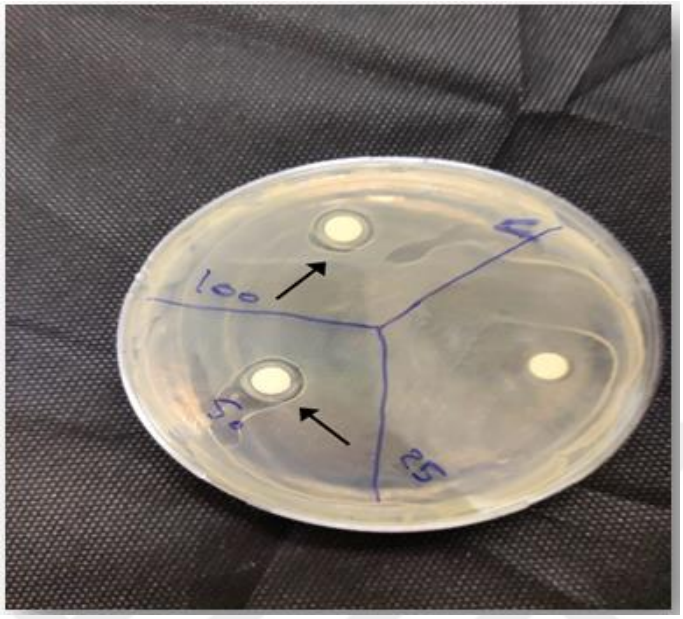


Figure 4.20. The biosurfactant produced by *B.velezensis* against *S.aureus* (nature)

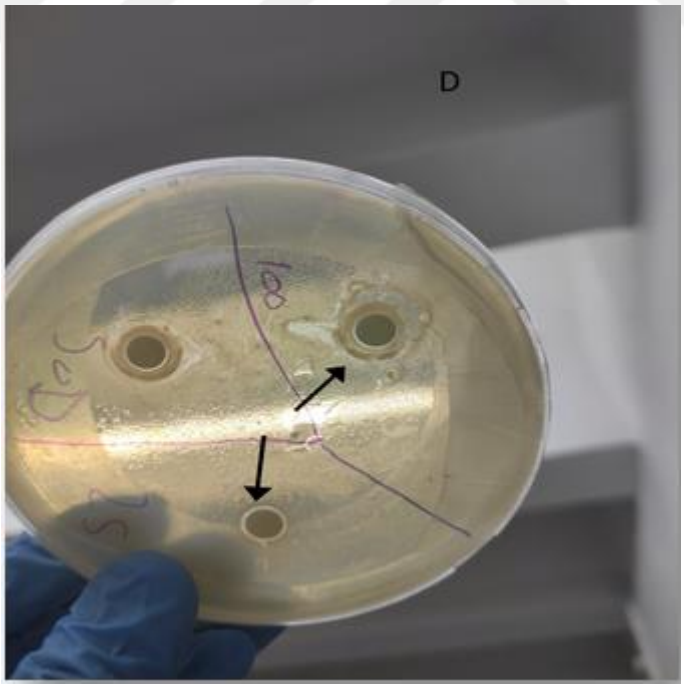


Figure 4.21. The biosurfactant produced by *B.velezensis* against *S.aureus* (Clinical isolate) 2

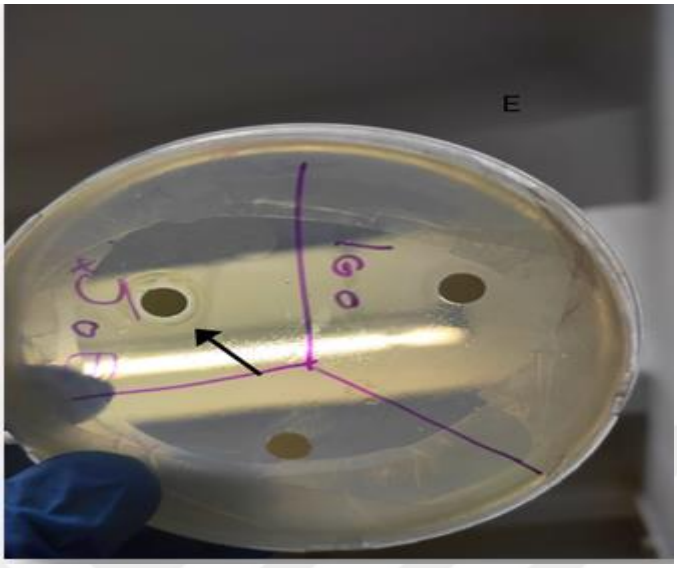


Figure 4.22. The biosurfactant produced by *B.velezensis* against *Salmonella spp*.

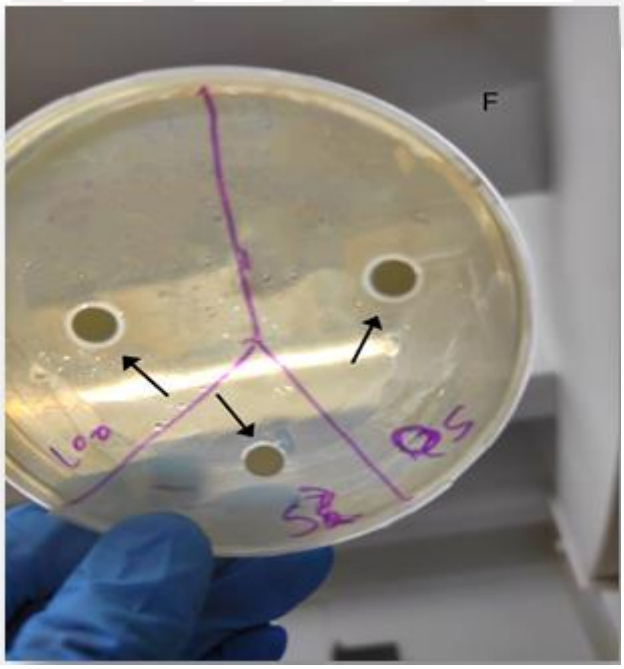


Figure 4.23. The biosurfactant produced by *B.velezensis* against *E.coli 1*

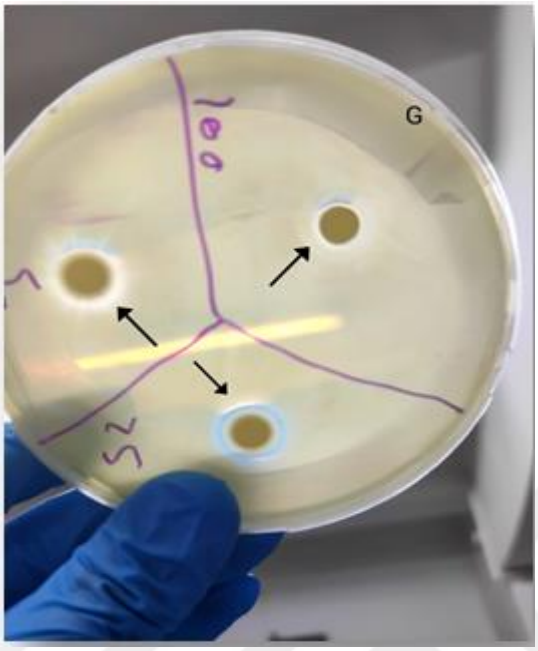


Figure 4.24. The biosurfactant produced by *B.velezensis* against *Klebsiella spp*.

Table 4.26. Antibacterial activity of the biosurfactant produced by *B.haynesii*

microorganism	pathogen	Biosurfactant Concentration		
		25 µL	50 µL	100µL
B.haynesii	*E.coli 2*	-	0.8 cm	1.0 cm
	S.aureus(Clinical isolate) 1	0.9 cm	1.1 cm	1.25 cm
	S.aureus (nature)	-	-	-
	S.aureus(Clinical isolate) 2	-	1.1 cm	1.35cm
	Salmonella spp.	-	0.8 cm	0.9 cm
	E.coli 1	0.85 cm	0.9 cm	0.95 cm
	Klebsiella spp.	0.85 cm	0.9 cm	0.9 cm
	*S.aureus*ATCC 29213	-	-	-

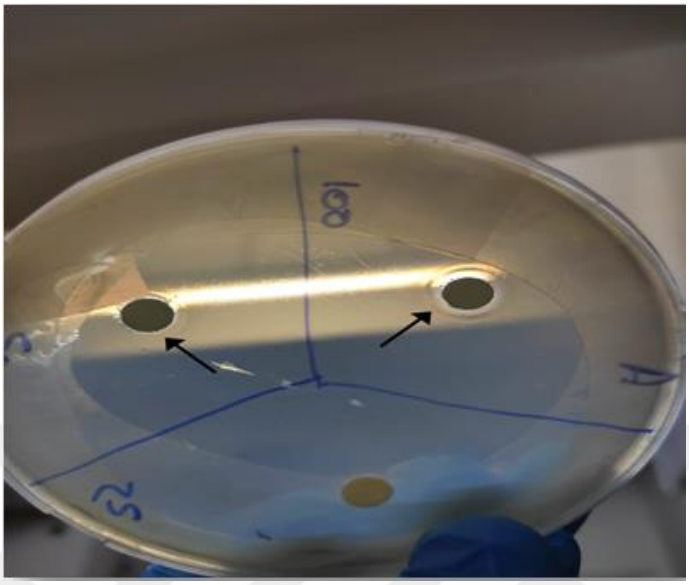


Figure 4.25. The biosurfactant produced by *B.haynesii* against *E.coli 2*

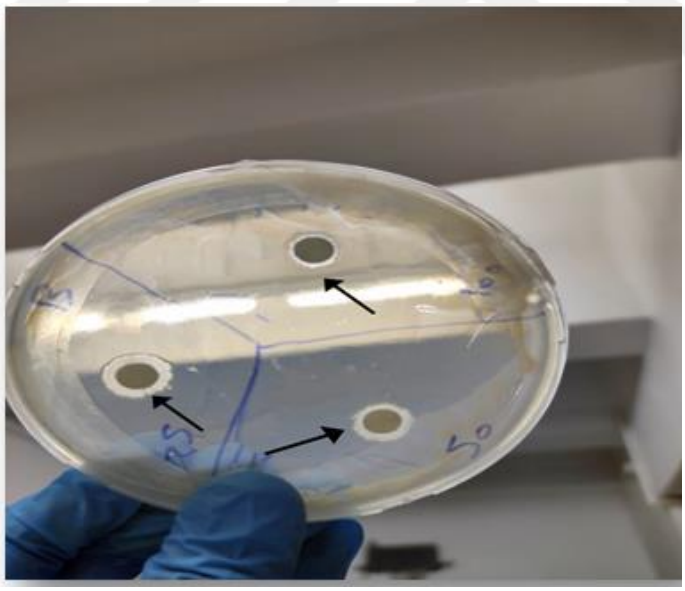


Figure 4.26. The biosurfactant produced by *B.haynesii* against *S.aureus* (Clinical isolate) 1

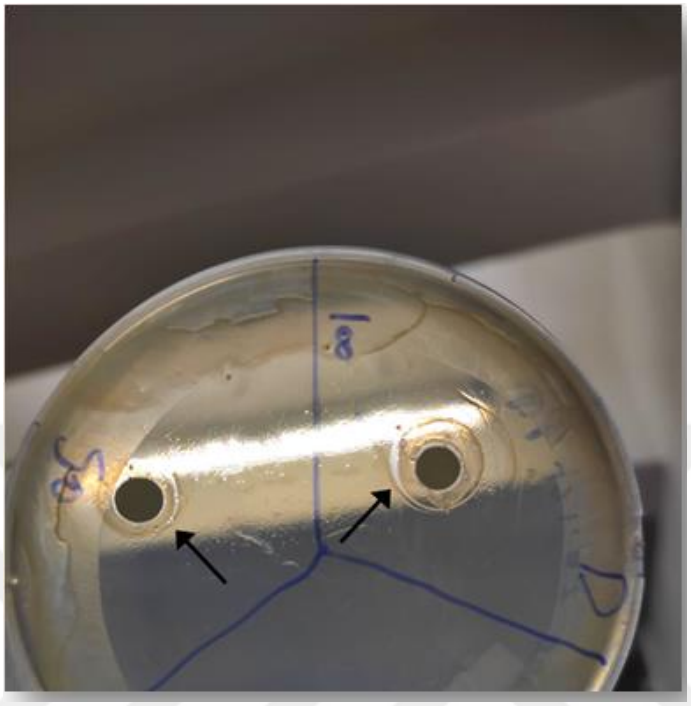


Figure 4.27. The biosurfactant produced by *B.haynesii* against *S.aureus* (Clinical isolate) 2

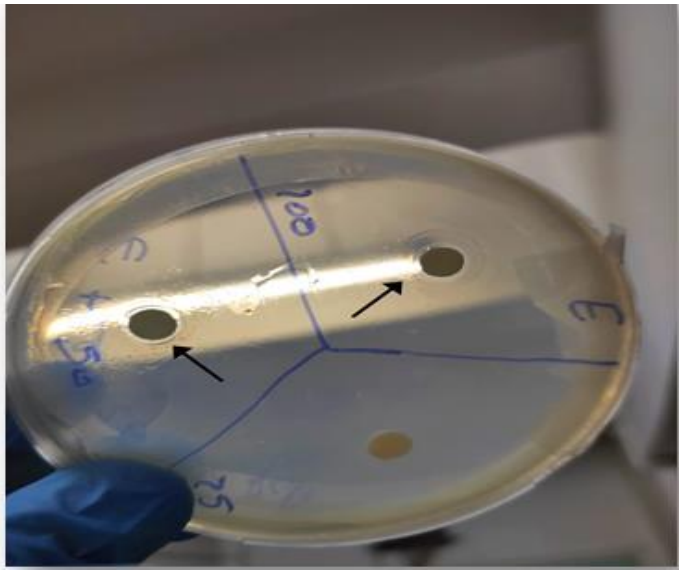


Figure 4.28. The biosurfactant produced by *B.haynesii* against *Salmonella spp*.

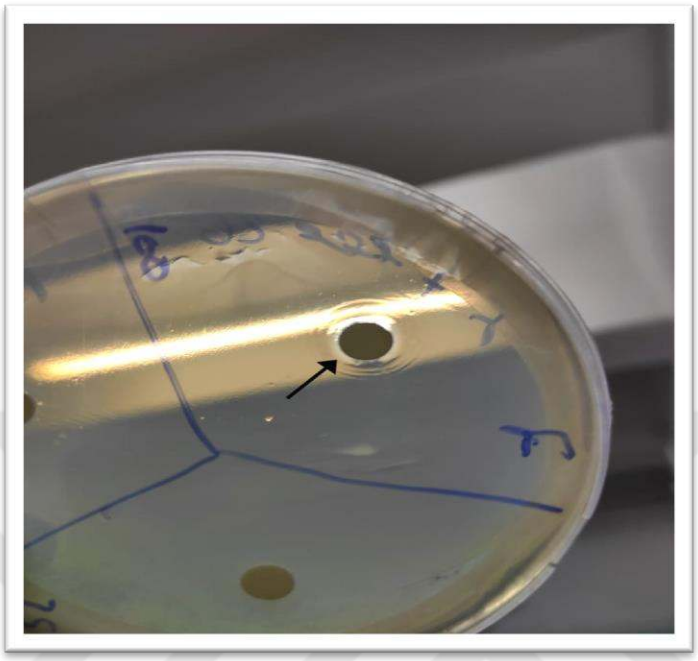


Figure 4.29. The biosurfactant produced by *B.haynesii* against *E.coli 1*

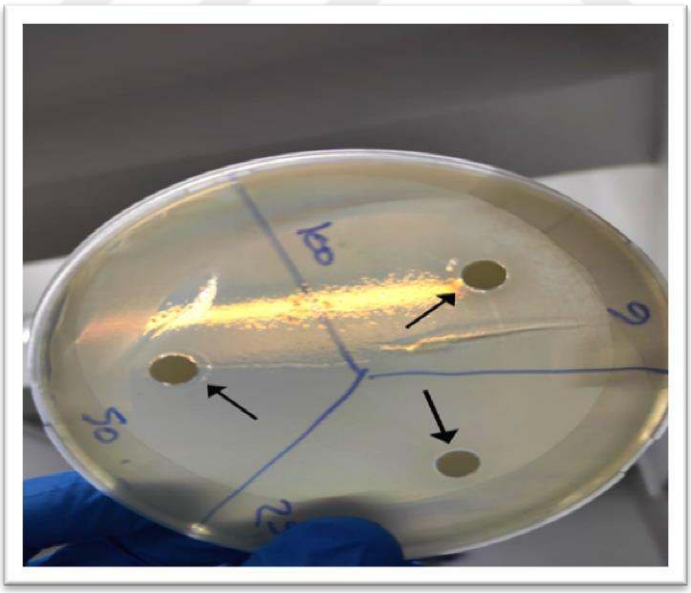


Figure 4.30. The biosurfactant produced by *B.haynesii* against *Klebsiella spp*.

Table 4.27. Antibacterial activity of the biosurfactant produced by *B.halotolerans*

microorganism	pathogen	Biosurfactant Concentration		
		25 µL	50 µL	100µL
B.halotolerans	*E.coli 2*	0.7 cm	-	0.8 cm
	S.aureus(Clinical isolate) 1	-	1.0 cm	1.1 cm
	S.aureus (nature)	-	-	1.2 cm
	S.aureus(Clinical isolate) 2	-	1.0 cm	1.3 cm
	Salmonella spp.	-	0.8 cm	1.0 cm
	E.coli 1	-	-	-
	Klebsiella spp.	0.6 cm	0.75 cm	0.8 cm
	*S.aureus*ATCC 29213	-	-	-

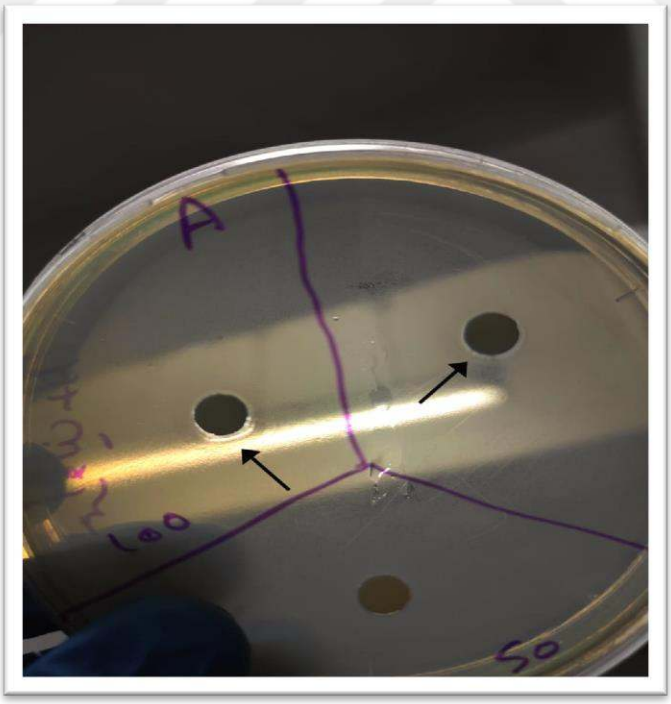


Figure 4.31. The biosurfactant produced by *B.halotolerans* against *E.coli 2*

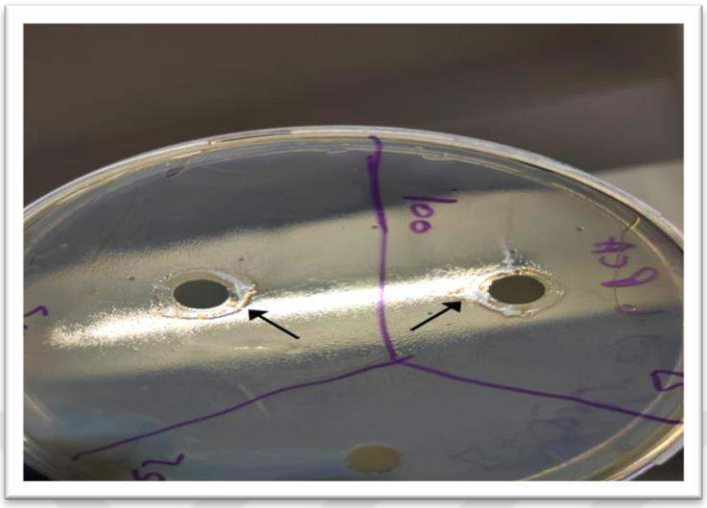


Figure 4.32. The biosurfactant produced by *B.halotolerans* against *S.aureus* (Clinical isolate) 1

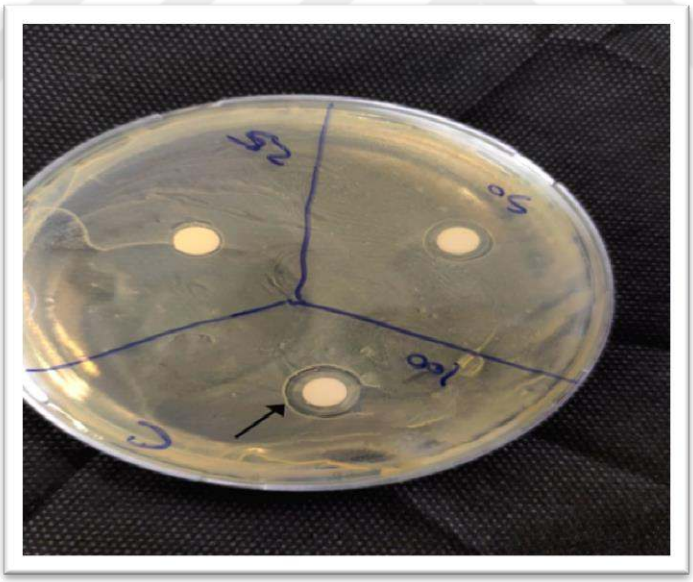


Figure 4.33. The biosurfactant produced by *B.halotolerans* against *S.aureus* (nature)

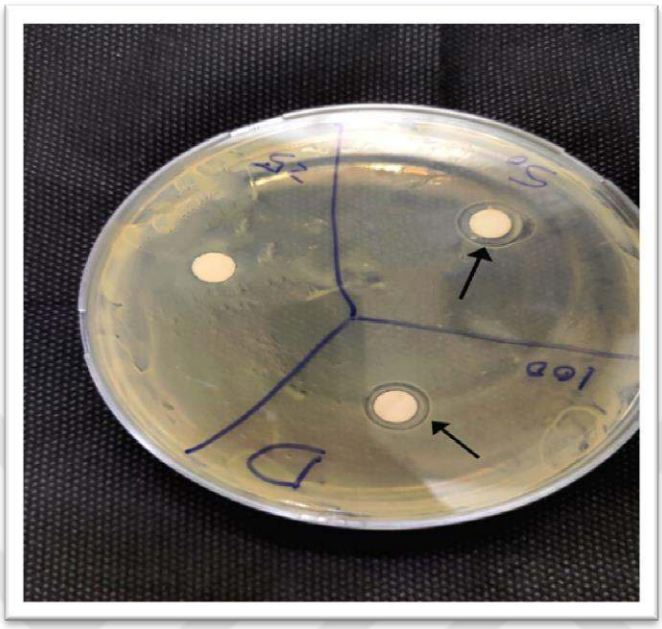


Figure 4.34. The biosurfactant produced by *B.halotolerans* against *S.aureus* (Clinical isolate) 2

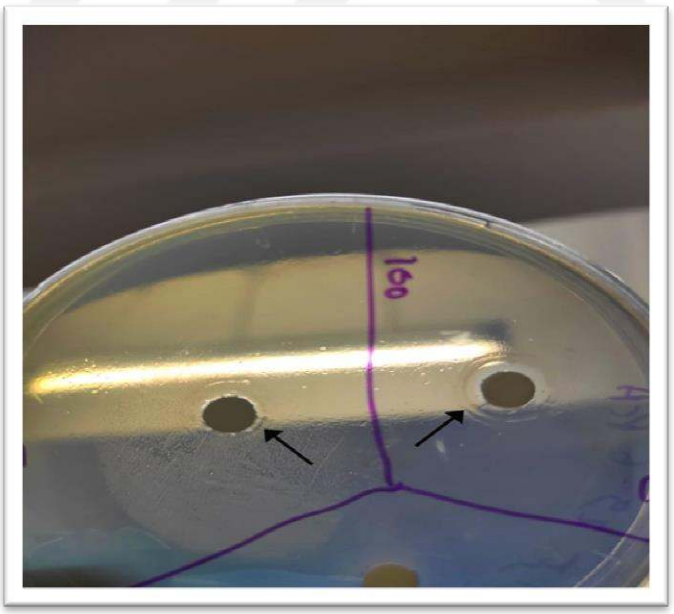


Figure 4.35. The biosurfactant produced by *B.halotolerans* against *Salmonella spp*.

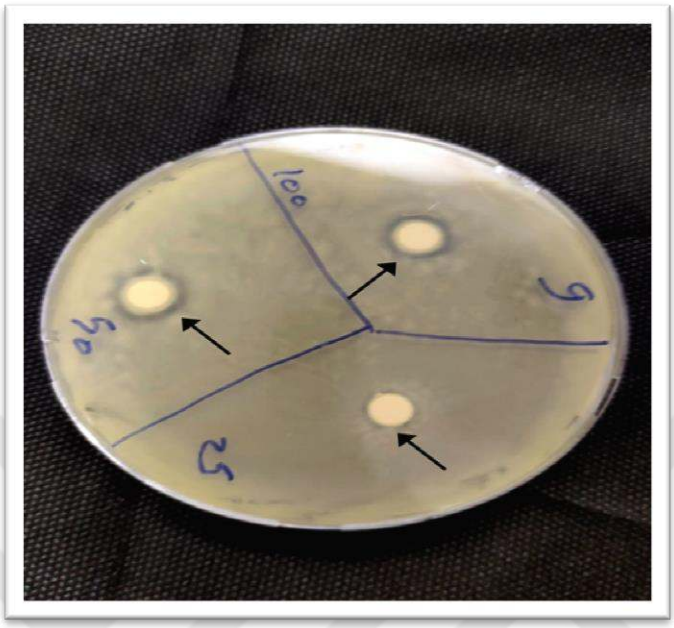


Figure 4.36. The biosurfactant produced by *B.halotolerans* against *Klebsiella spp*.

Table 4.28. Antibacterial activity of the biosurfactant produced by *P.reinekei*

microorganism	pathogen	Biosurfactant Concentration		
		25 µL	50 µL	100µL
P.reinekei	*E.coli 2*	-	-	0.9 cm
	S.aureus(Clinical isolate) 1	-	-	0.9 cm
	S.aureus (nature)	-	-	0.85 cm
	S.aureus(Clinical isolate) 2	-	-	1.1 cm
	Salmonella spp.	-	0.9 cm	1.0 cm
	E.coli 1	-	0.8 cm	0.85 cm
	Klebsiella spp.	-	1.0 cm	1.1 cm
	S.aureusATCC 29213	-	-	-

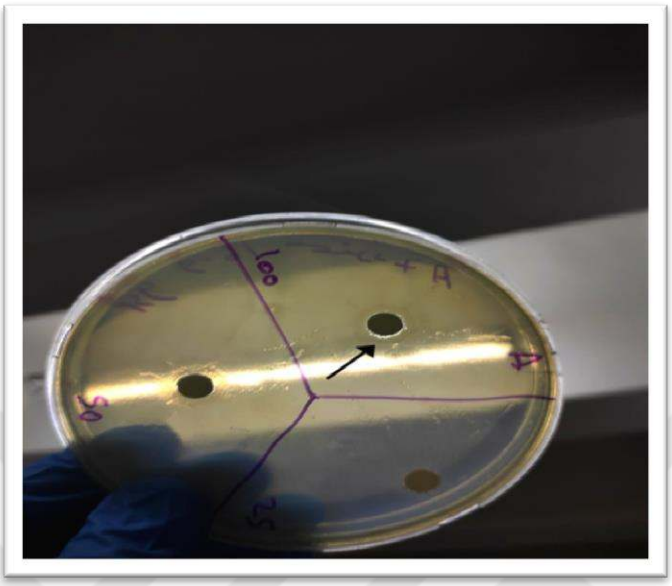


Figure 4.37. The biosurfactant produced by *P.reinekei* against *E.coli 2*

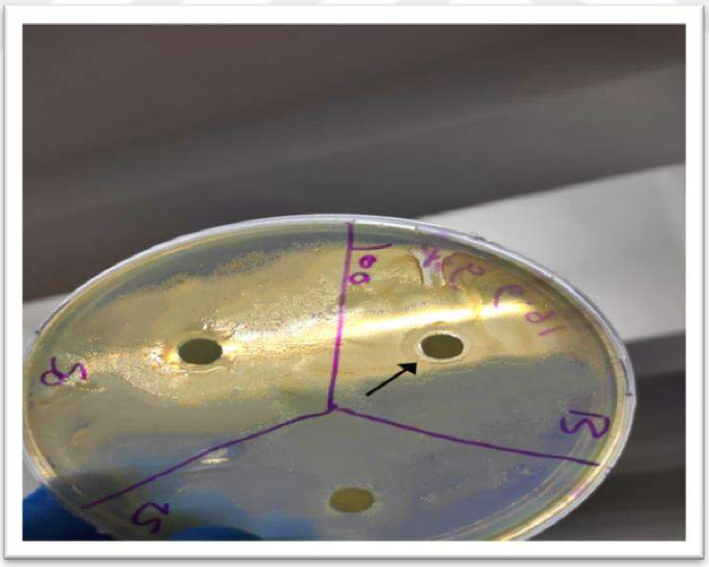


Figure 4.38. The biosurfactant produced by *P. reinekei* against *S.aureus* (Clinical isolate) 1

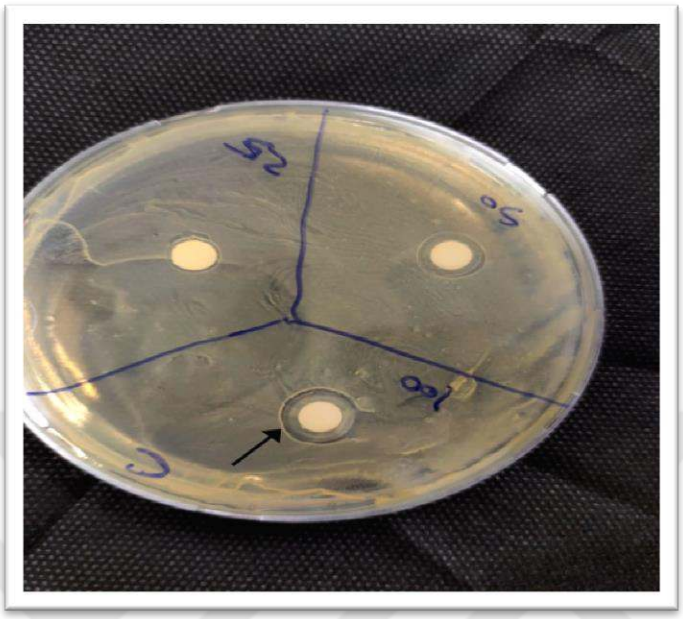


Figure 4.39. The biosurfactant produced by *P.reinekei* against *S.aureus* (nature)

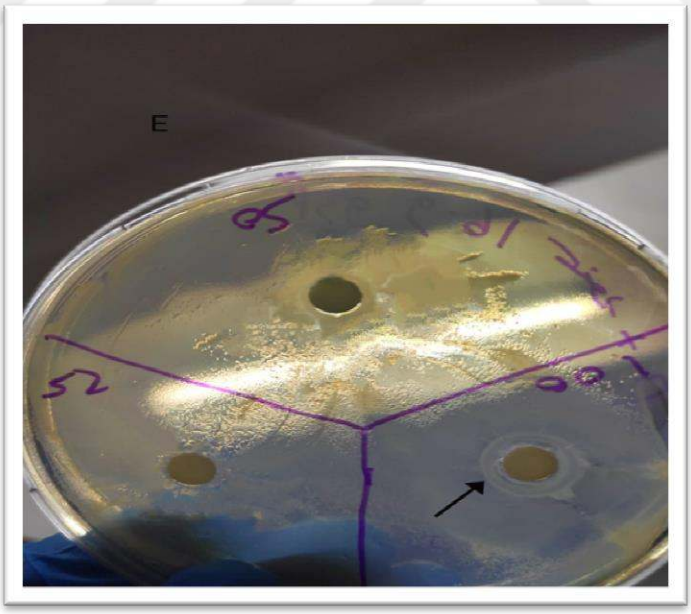


Figure 4.40. The biosurfactant produced by *P.reinekei* against *S.aureus* (Clinical isolate) 2

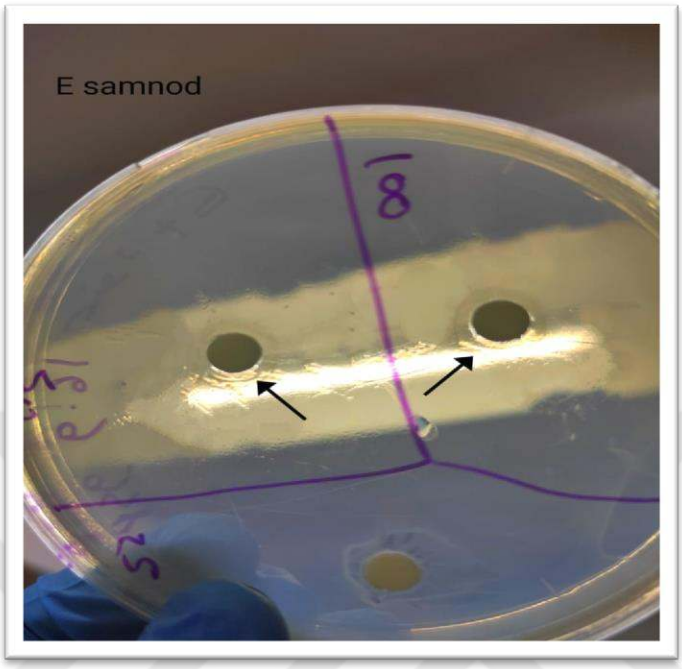


Figure 4.41. The biosurfactant produced by *P.reinekei* against *Salmonella spp*.

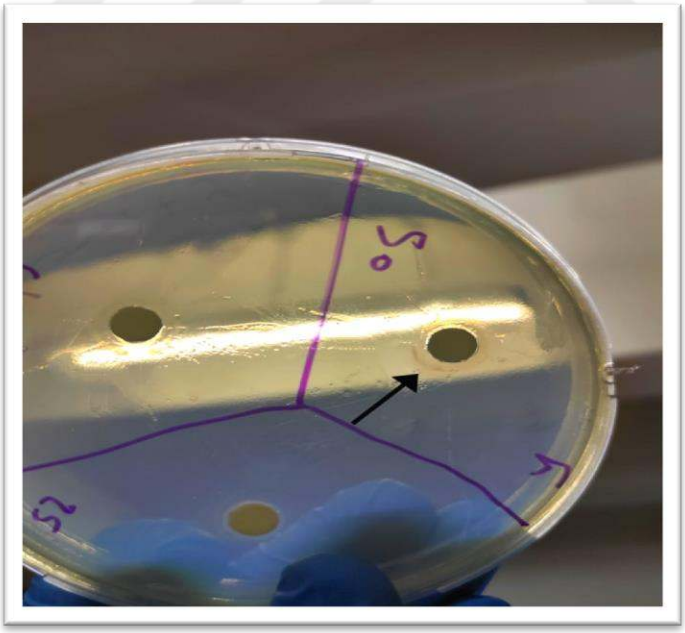


Figure 4.42. The biosurfactant produced by *P.reinekei* against *E.coli 1*

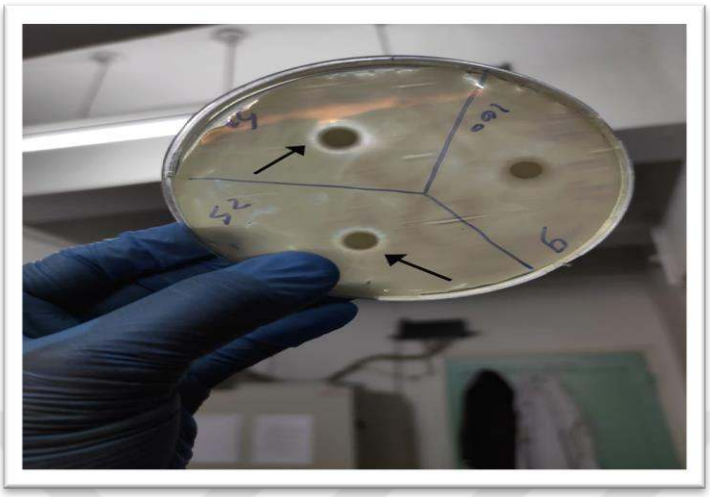


Figure 4.43. The biosurfactant produced by *P.reinekei* against *Klebsiella spp*.

Table 4.29. Antibacterial activity of the biosurfactant produced by *M.arborescens*

microorganism	pathogen	Biosurfactant Concentration		
		25 µL	50 µL	100µL
M.arborescens	*E.coli 2*	-	-	1.0 cm
	S.aureus(Clinical isolate) 1	-	2.0 cm	1.75 cm
	S.aureus (nature)	-	1.25 cm	1.35 cm
	S.aureus(Clinical isolate) 2	-	1.2 cm	1.4 cm
	Salmonella spp.	-	-	1.0 cm
	E.coli 1	-	0.8 cm	0.8 cm
	Klebsiella spp.	0.75 cm	0.8 cm	0.9 cm
	S.aureusATCC 29213	0.85 cm	-	-

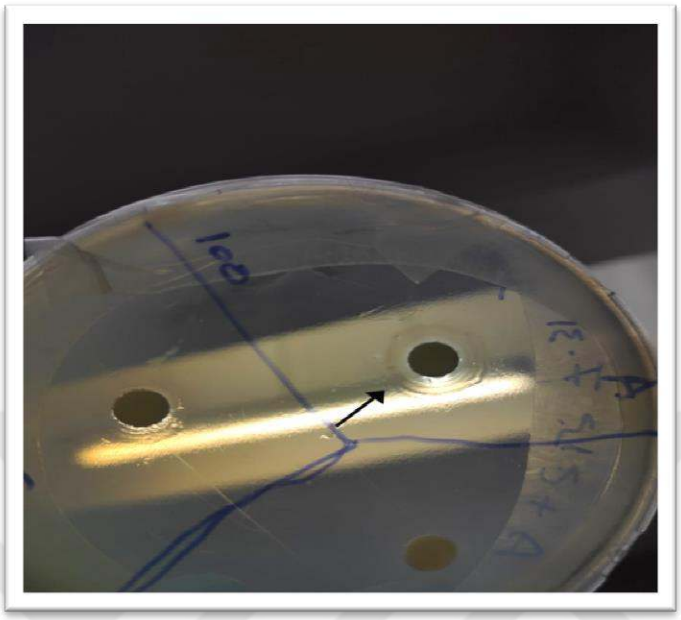


Figure 4.44. The biosurfactant produced by *M.arborescens* against *E.coli 2*

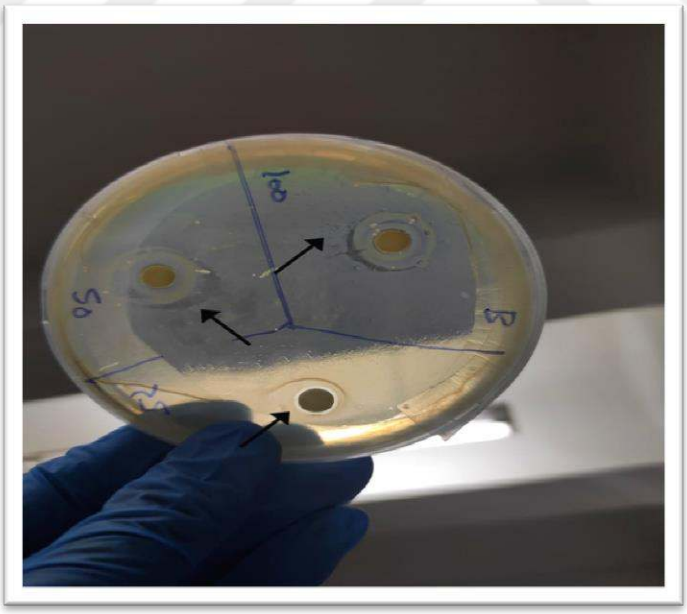


Figure 4.45. The biosurfactant produced by *M.arborescens* against *S.aureus* (Clinical isolate) 1

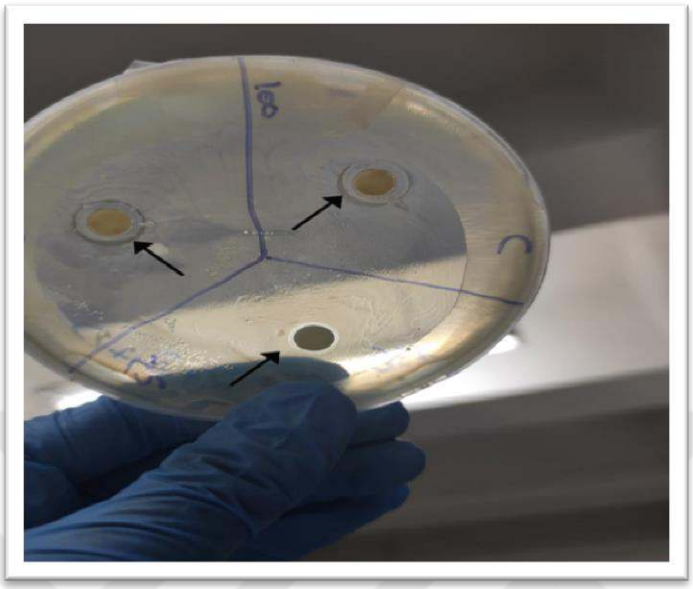


Figure 4.46. The biosurfactant produced by *M.arborescens* against *S.aureus* (nature)

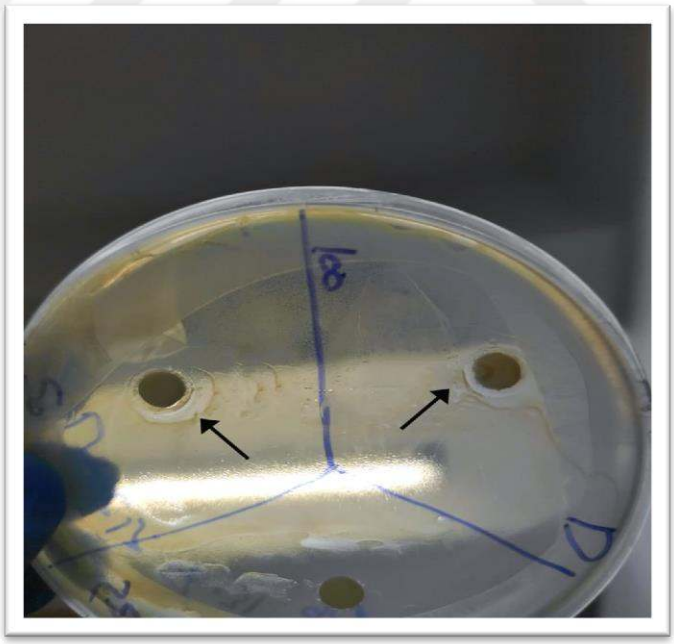


Figure 4.47. The biosurfactant produced by *M.arborescens* against *S.aureus* (Clinical isolate) 2

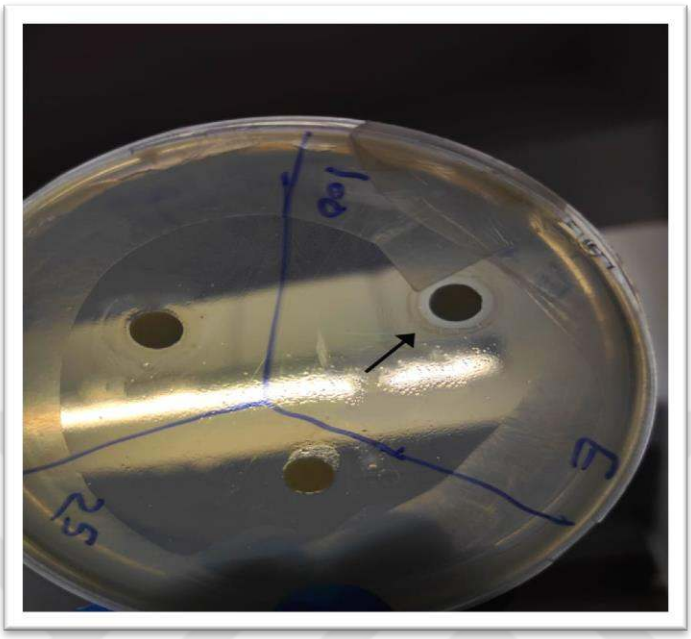


Figure 4.48. The biosurfactant produced by *M.arborescens* against *Salmonella spp*.

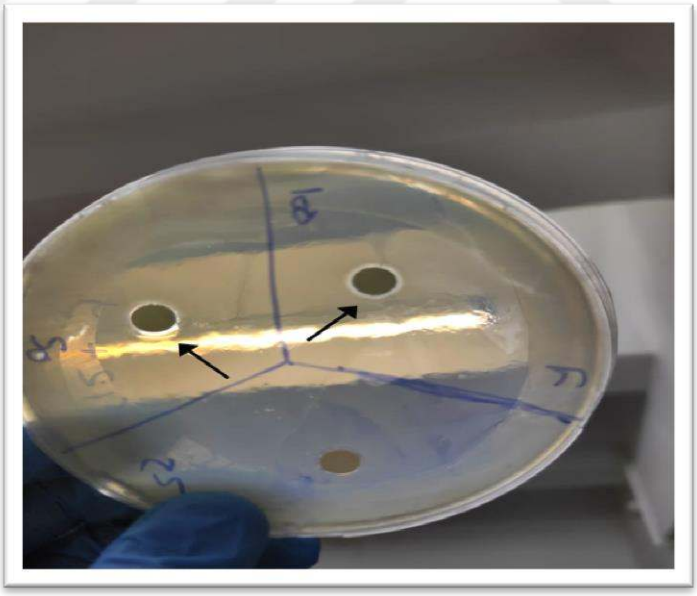


Figure 4.49. The biosurfactant produced by *M.arborescens* against *E.coli 1*

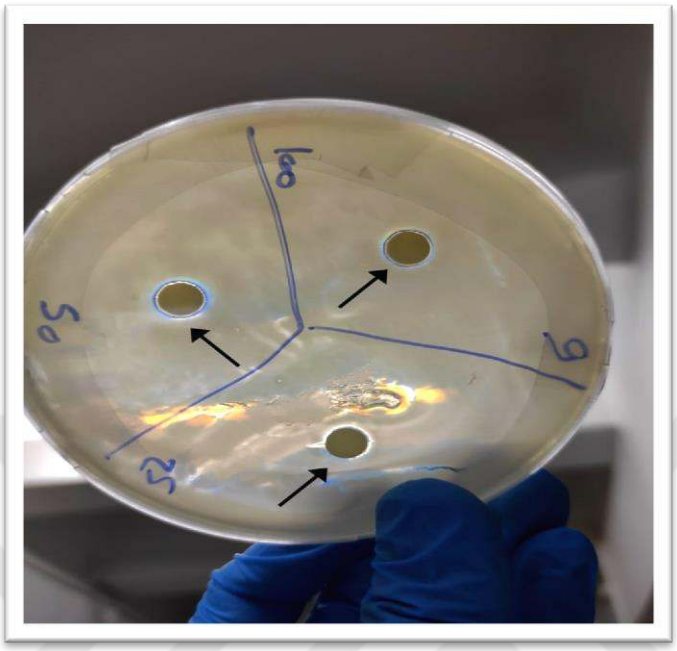


Figure 4.50. The biosurfactant produced by *M.arborescens* against *Klebsiella spp*.

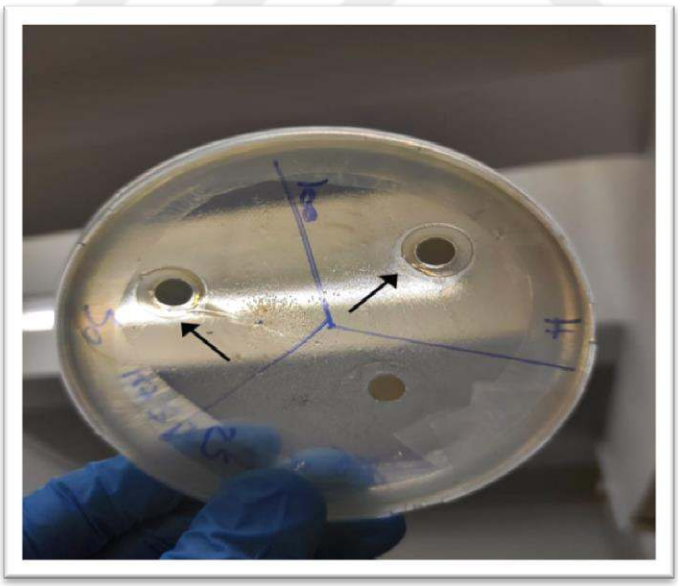


Figure 4.51. The biosurfactant produced by *M.arborescens* against *S.aureus ATCC 29213*

Table 4.30. Antibacterial activity of the biosurfactant produced by *P.veronii*

microorganism	pathogen	Biosurfactant Concentration		
		25 µL	50 µL	100µL
P.veronii	*E.coli 2*	-	1.2 cm	1.0 cm
	S.aureus(Clinical isolate) 1	-	-	-
	S.aureus (nature)	-	-	-
	S.aureus(Clinical isolate) 2	-	-	-
	Salmonella spp.	1.0 cm	1.0 cm	1.0 cm
	E.coli 1	-	0.9 cm	1.0 cm
	Klebsiella spp.	0.75 cm	0.8 cm	0.9 cm
	S.aureusATCC 29213	-	-	-

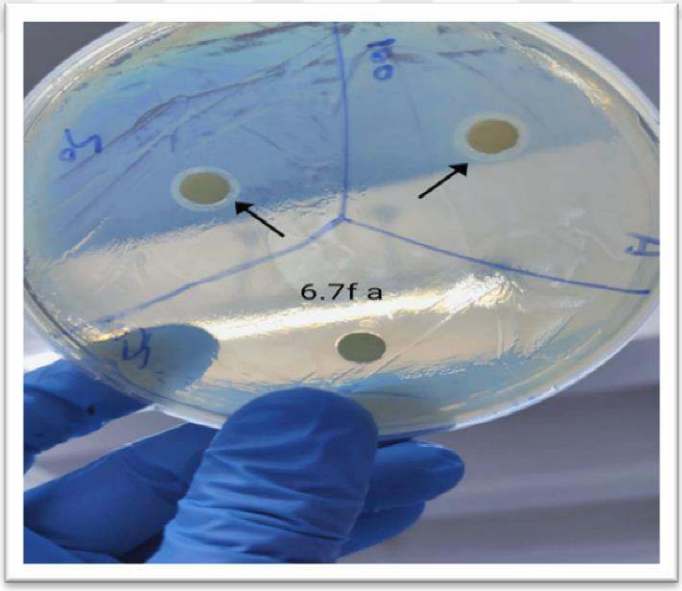


Figure 4.52. The biosurfactant produced by *P.veronii* against *E.coli 2*

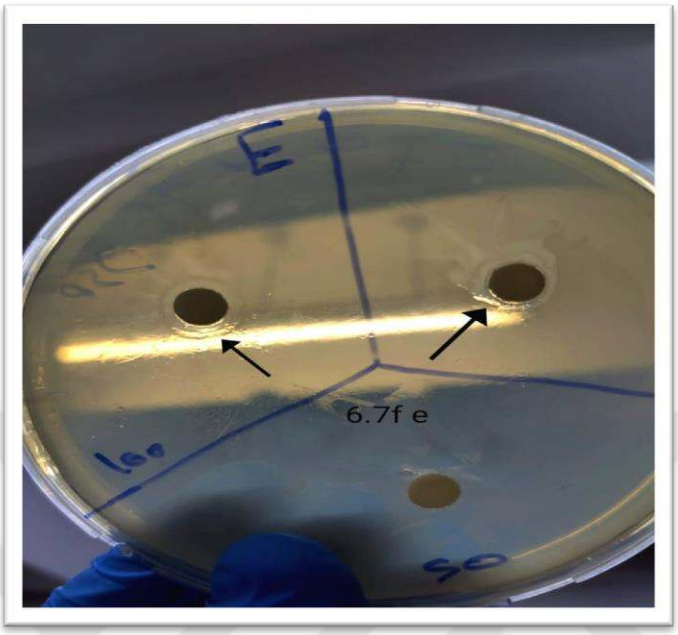


Figure 4.53. The biosurfactant produced by *P.veronii* against *Salmonella spp*.

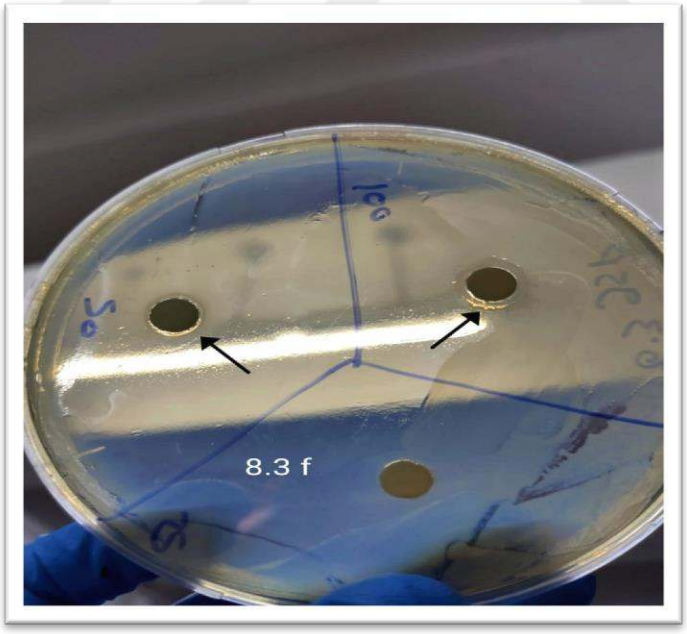


Figure 4.54. The biosurfactant produced by *P.veronii* against *E.coli 1*

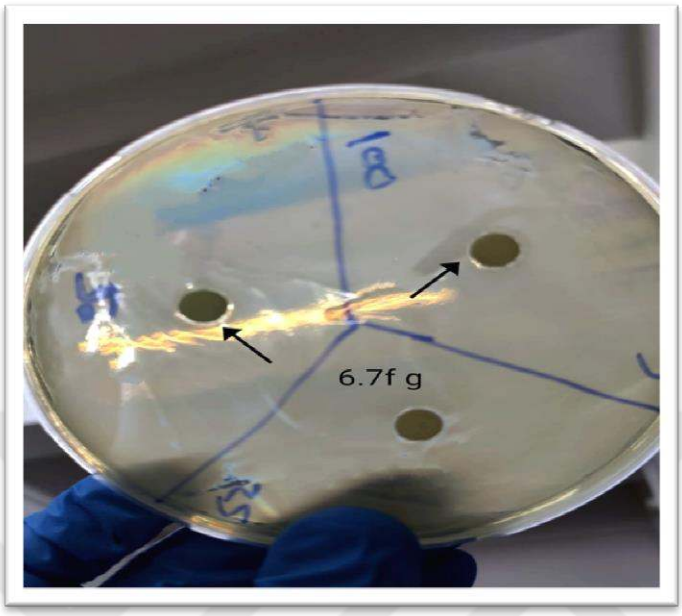


Figure 4.55. The biosurfactant produced by *P.veronii* against *Klebsiella spp*.

Table 4.31. Antibacterial activity the biosurfactant produced by of *A.lwoffii*

microorganism	pathogen	Biosurfactant Concentration		
		25 µL	50 µL	100µL
A.lwoffii	E.coli 2	-	-	-
	S.aureus(Clinical isolate) 1	-	-	-
	S.aureus (nature)	-	-	-
	S.aureus(Clinical isolate) 2	-	-	-
	Salmonella spp.	-	-	1.0 cm
	E.coli 1	-	-	-
	Klebsiella spp.	0.8 cm	0.9 cm	-
	S.aureusATCC 29213	-	-	-

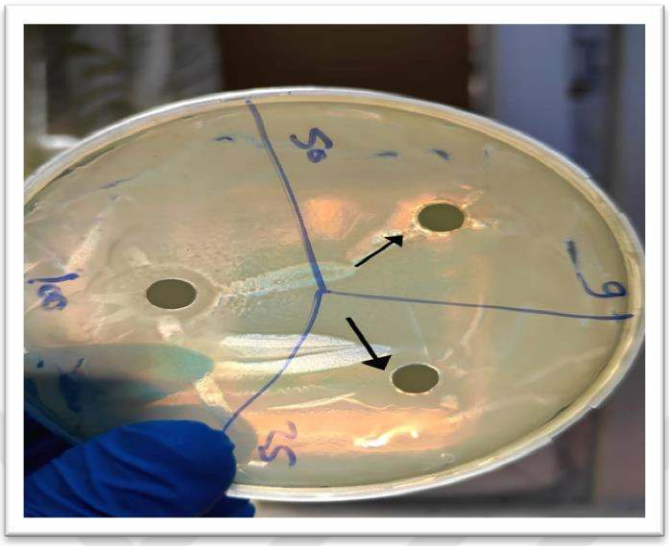


Figure 4.56. The biosurfactant produced by *A.lwoffii* against *Klebsiella spp*.

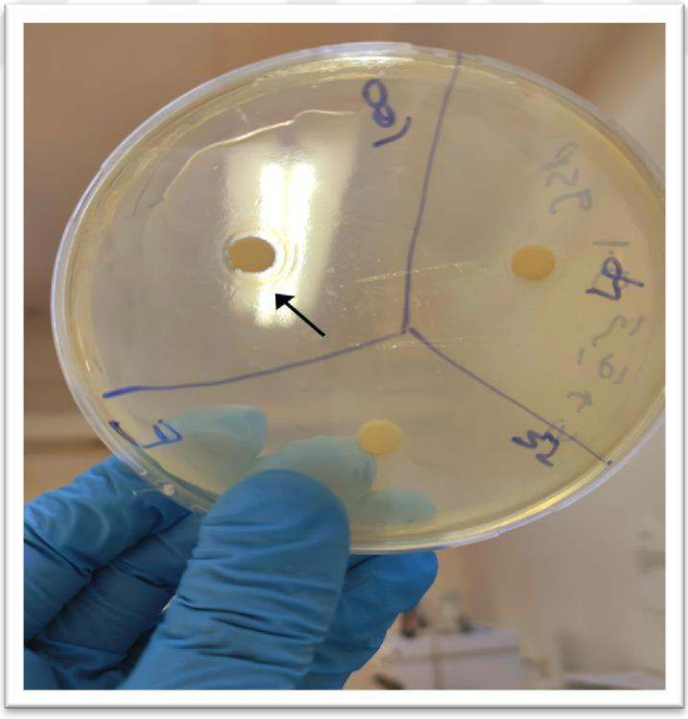
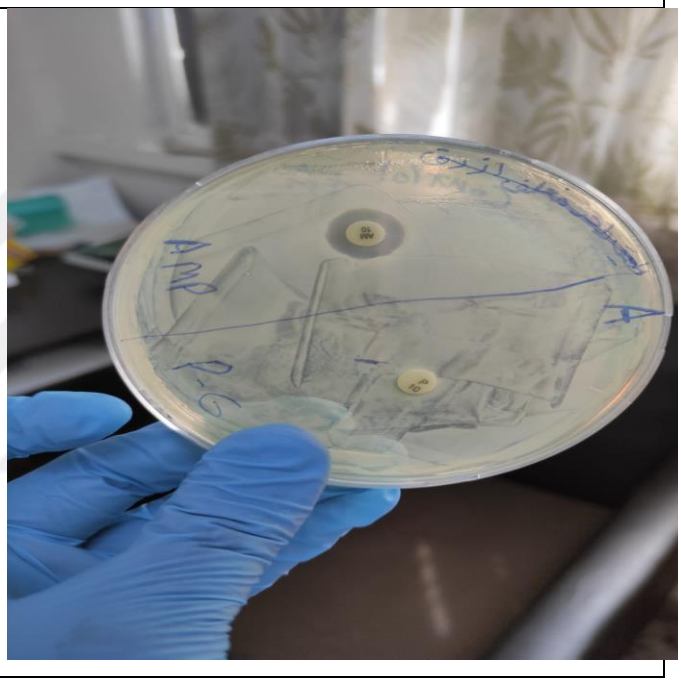
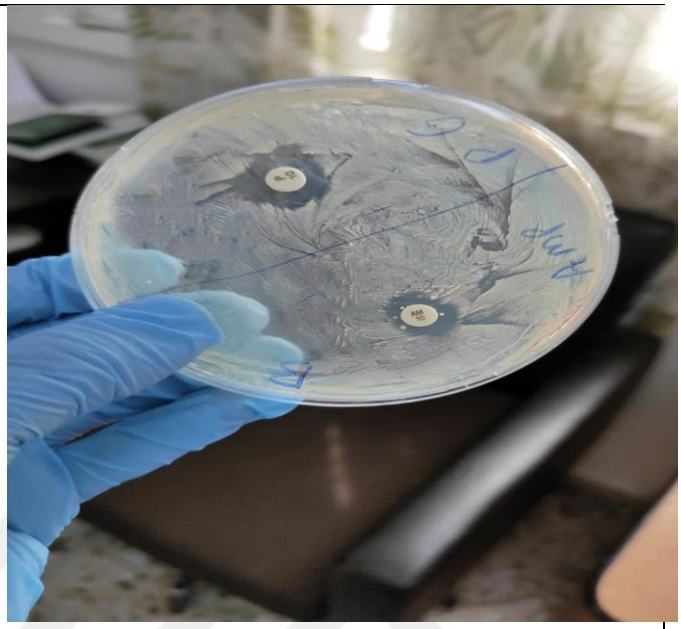
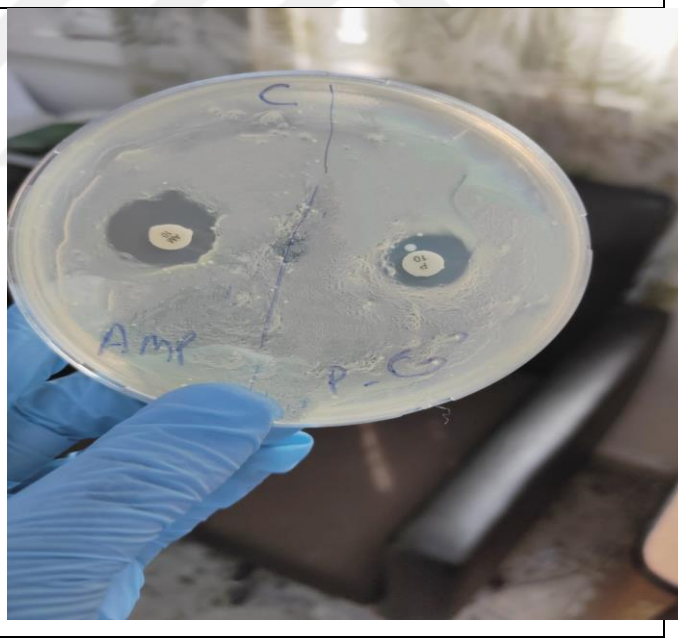
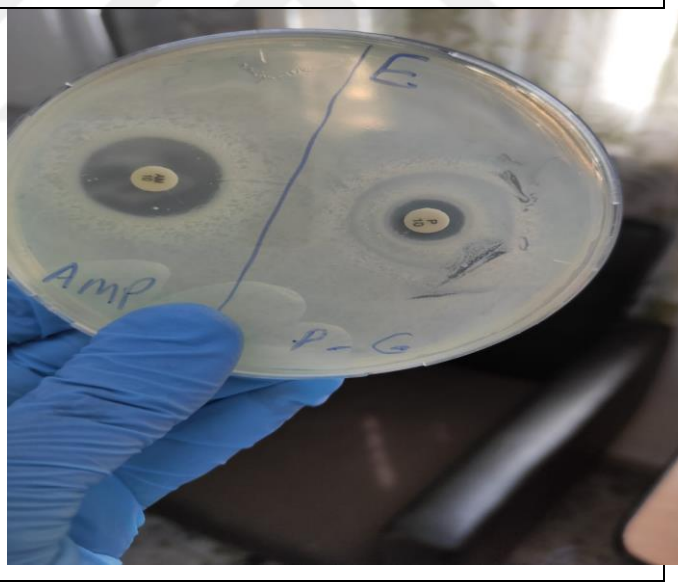


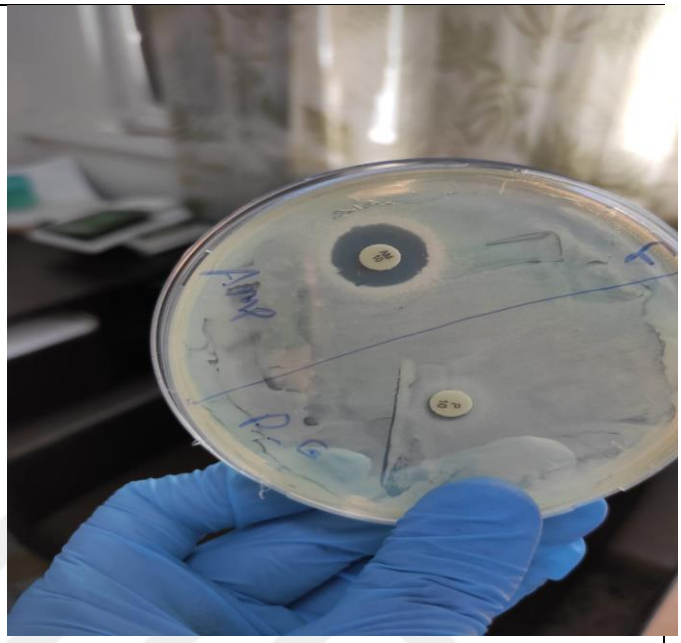
Figure 4.57. The biosurfactant produced by *A.lwoffii* against *Salmonella spp*.

Table 4.32. Antibacterial activity of penicillin *G* and Ampicillin as a positive control in the antibacterial assay.

pathogen	Positive control		Figure
	penici-llin G	*Ampicillin*	
E.coli 2	-	1.2 cm	

S.aureus (Clinical isolate) 1	1.5 cm	1.1 cm	
S.aureus (nature)	1.2 cm	1.65 cm	

S.aureus (Clinical isolate) 2	1.25 cm	1.25 cm	
Salmonella spp.	1.1 cm	1.9 cm	

E.coli 1	0.6 cm	1.5 cm	
Klebsiella spp.	0.7 cm	0.8 cm	

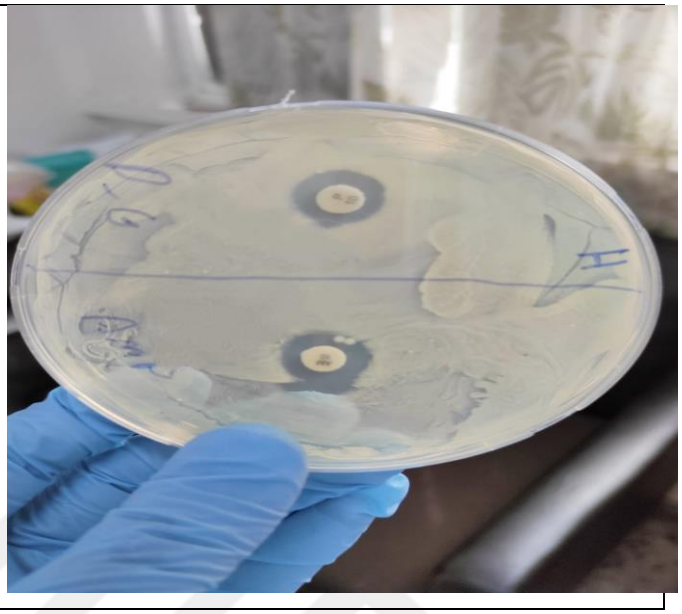
S.aureus ATCC 29213	1.2 cm	1.3 cm	

Figure 4.58. Antimicrobial activity (25 µL)

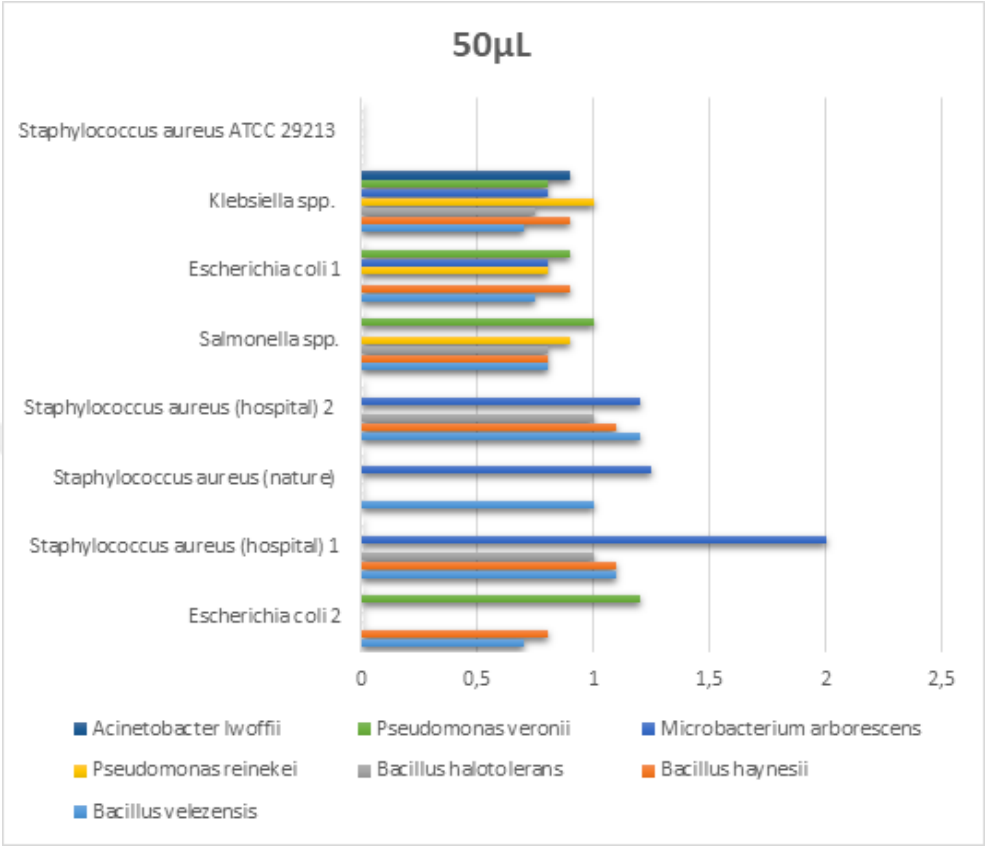


Figure 4.59. Antimicrobial activity (50 µL)

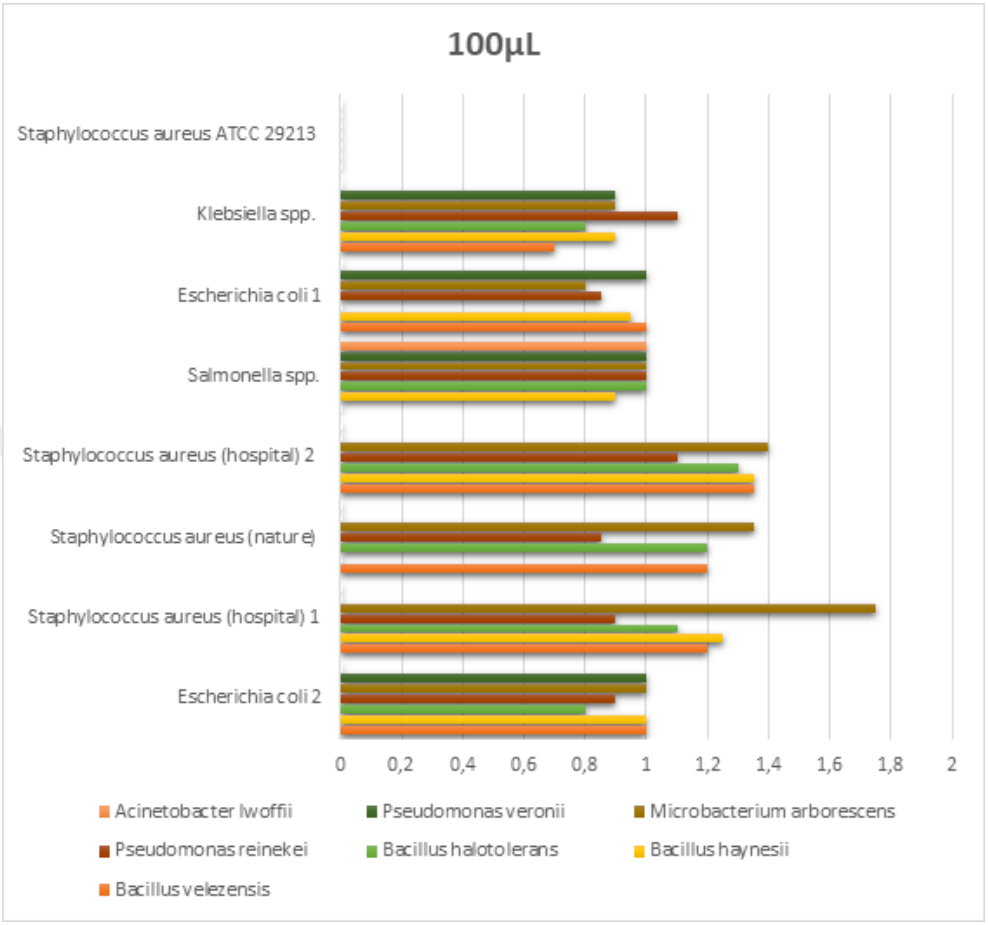
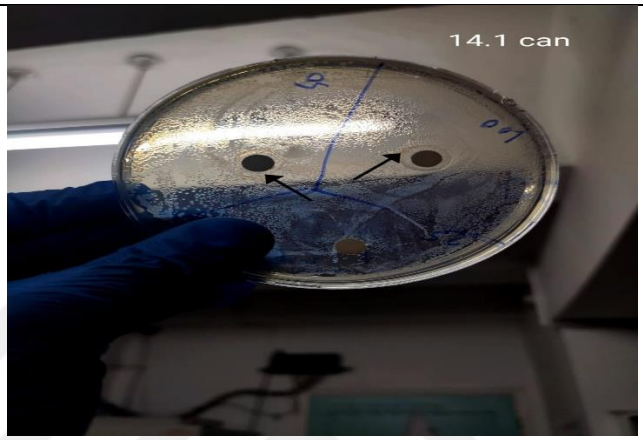
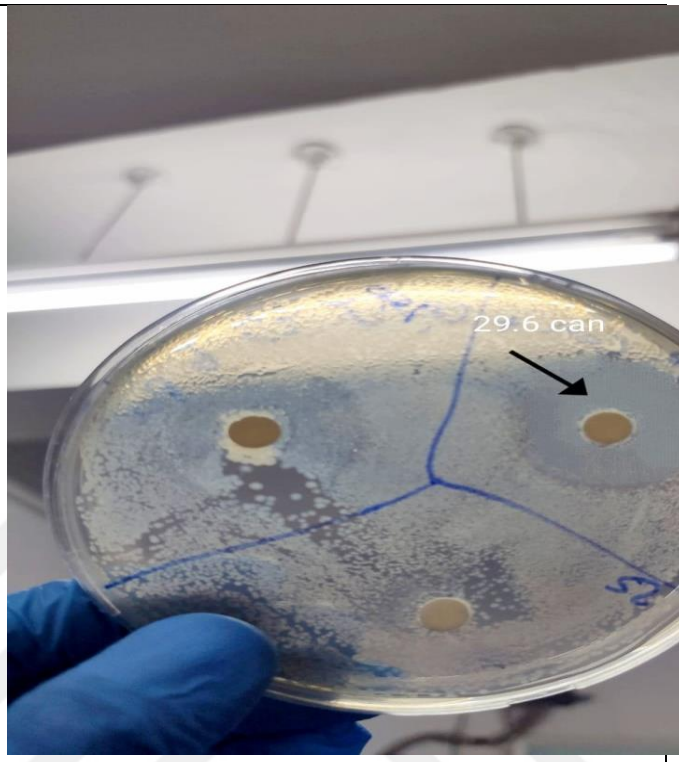
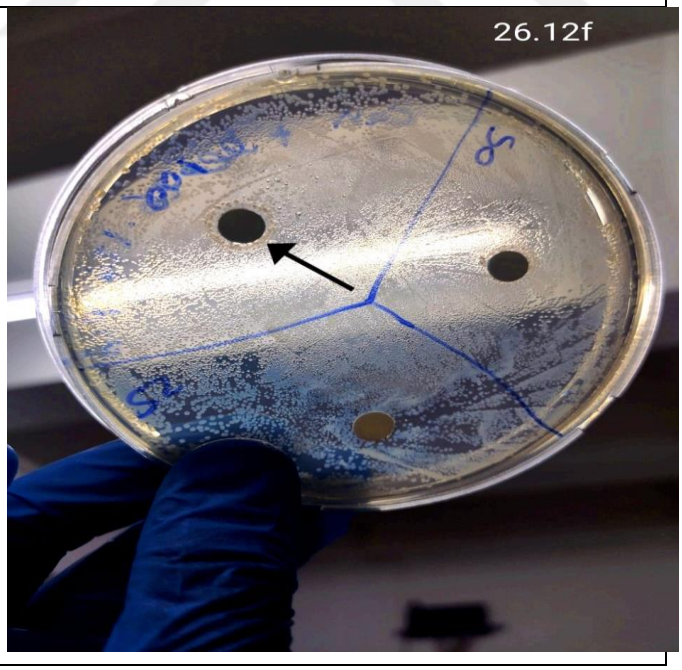
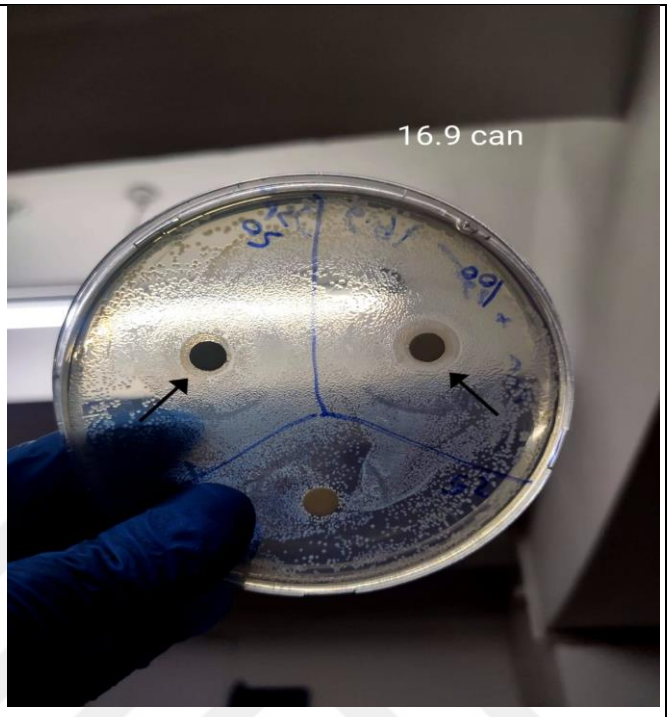
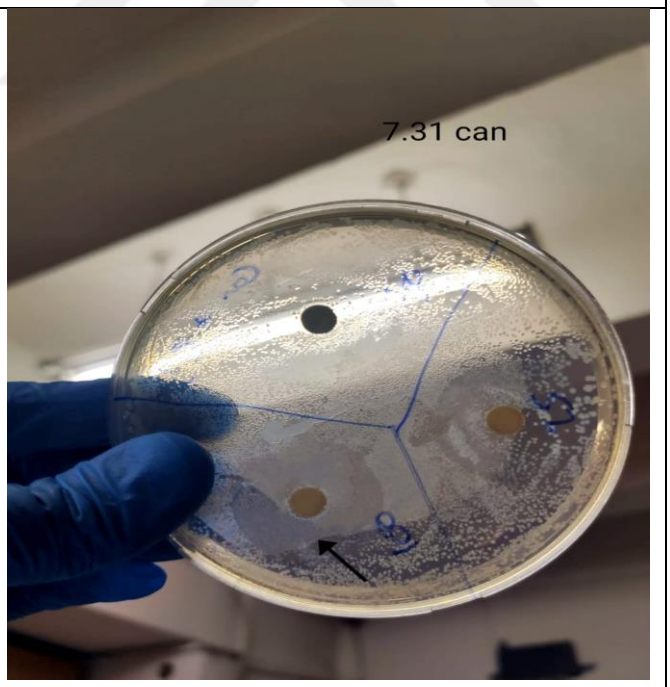


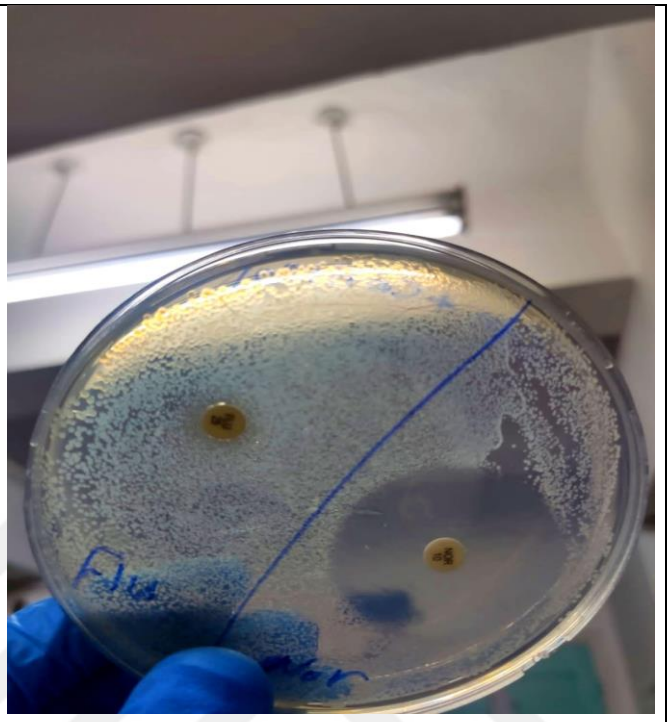
Figure 4.60. Antimicrobial activity (100 µL)

Table 4.33. Antifungal activity of the biosurfactant produced by isolated bacteria against *C.albicans* and Norfloxacin as a positive control

microorganism	the zone diameter around the discs	Figure
B.velezensis	50 µL 0.9 cm	
	100µL 1.0 cm	

B.halotolerans	100 µL 2.4 cm	
B.haynesii	100 µL 0.9 cm	

<table>
<tr><td rowspan="2">P.reinekei</td><td>50 µL
1.0 cm</td><td rowspan="2"> 
</td></tr>
<tr><td>100 µl
1.2 cm</td></tr>
<tr><td>M.arborescens</td><td>100 µL
2.5 cm</td><td> 
</td></tr>
</table>

Positive control (*Norfloxacin*)	3.7 cm	

4.6. Structural Characterization of Biosurfactants

4.6.1. Fourier Transformation Infra-Red (FTIR) Analysis

FTIR representations of biosurfactant-produced microbes are displayed in (Figure 4.58) broadband at 3300 cm−1indicated the presence of hydrogen bond with OH functional groups (Datta et al. 2018). The peaks at 1600 cm−1 showed the presence of C═C bonds of the aromatic group. The significant bond at 1047 cm−1 showed the availability of functional groups such as alkoxy groups (Datta et al. 2018). These functional groups in the FTIR analysis helped to predict the presence of fatty acid derivative. Therefore, it is possible that the extracted biosurfactant is a type of fatty acid biosurfactants.

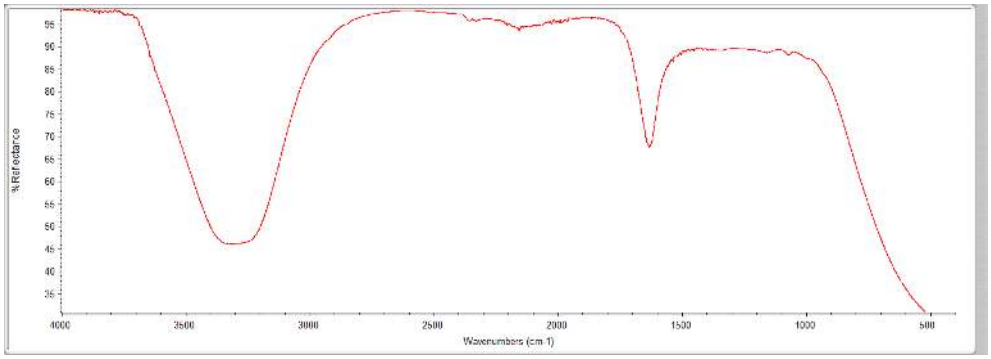


Figure 4.61. Fourier Transformation Infra-Red (FTIR) analysis of *B.velezensis*

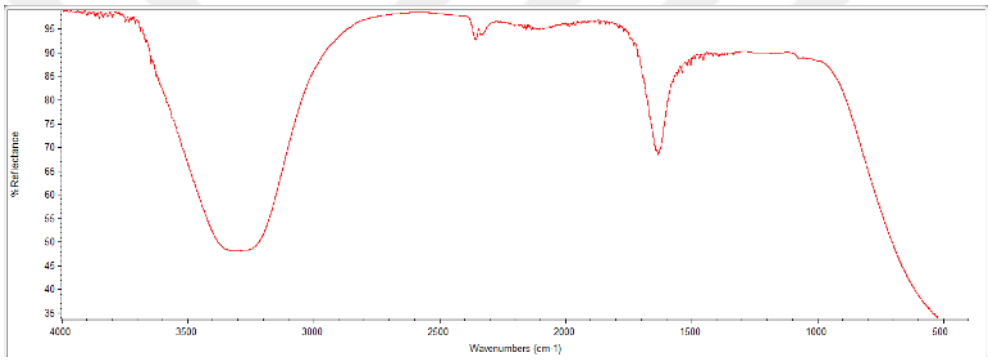


Figure 4.62. Fourier Transformation Infra-Red (FTIR) analysis of *B.haynesii*

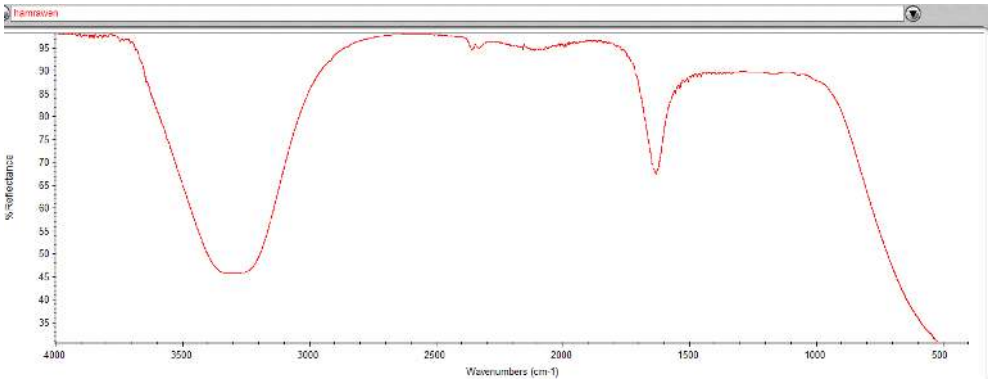


Figure 4.63. Fourier Transformation Infra-Red (FTIR) analysis of *B.halotolerans*

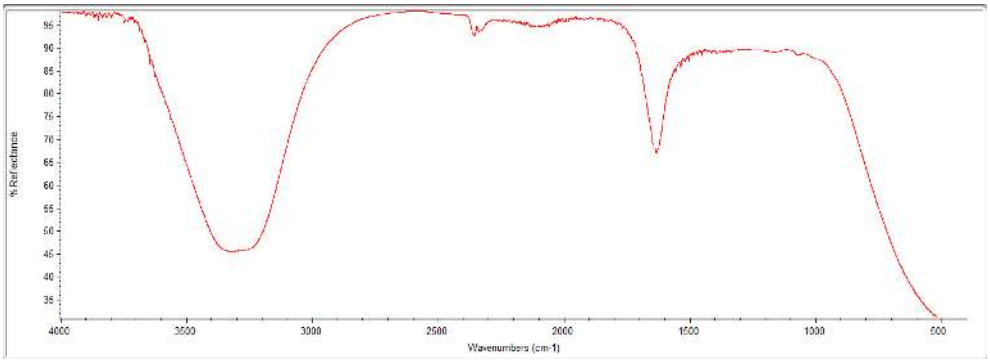


Figure 4.64. Fourier Transformation Infra-Red (FTIR) analysis of *P.reinekei*

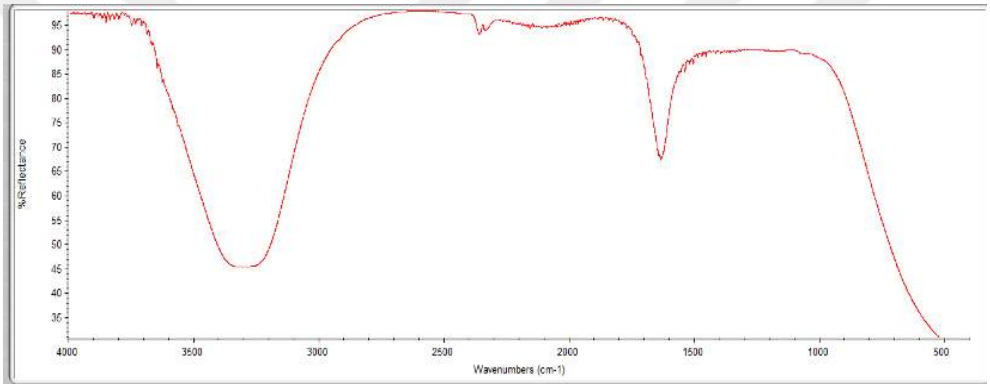


Figure 4.65. Fourier Transformation Infra-Red (FTIR) analysis of *M.arborescens*

4.6.2. NMR Spectroscopy

4.6.2.1. 1H NMR Analysis

Since there are peaks between 0.5-1.5 ppm, there are alkyls in the structure, The peak at 2,5 ppm is an indication of the aromatic ring (it may also be an alkene indicator). The peak at 3.5 ppm is the hydrogen indicator bound to the oxygen (there are alcohol groups in the structure) The peaks between 6.5-7.5 ppm indicates a low probability of the presence of aromatic ring in the structure and the Peaks between 8-8.5 ppm may be an indication that there is an aldehyde in the structure. Finally, according to the peaks at 0.85, 1.2 and 1.5 ppm there is a probability that the molecule is a fatty acid derivative. Therefore, it is possible that the extracted biosurfactant is a type of fatty acid biosurfactants.

4.6.2.2. 13C NMR Spectra Analysis

The peak obtained at 40 ppm is indicative of saturated carbon atoms. Peaks are difficult to separate because of the high hydrogen coupling. This peak may also indicate $H_2CC{=}O$ carbons.

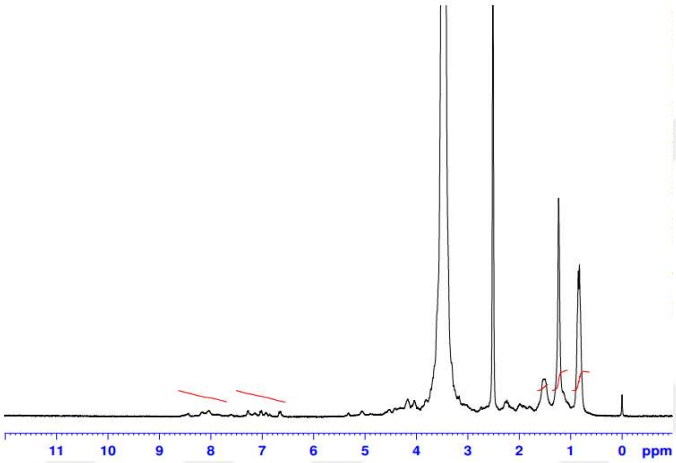


Figure 4.66. 1H NMR Analysis

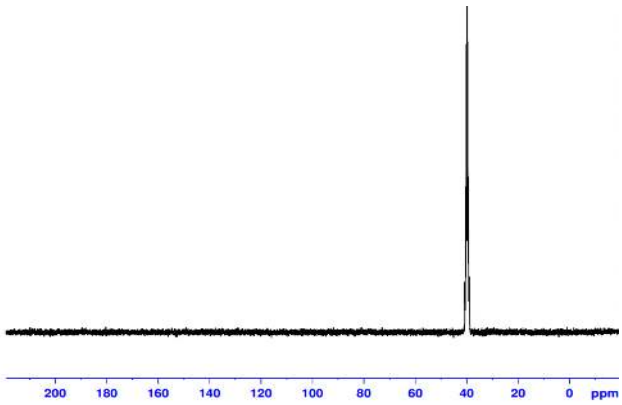


Figure 4.67. 13C NMR Spectra Analysis

5. CONCLUSION AND RECOMMENDATIONS

Environmental pollution is not a new phenomenon, but it remains the world's most serious problem and one of the leading causes of illness and mortality. Urbanization, industrialization, mining, and exploration are all examples of human activities that contribute to global environmental pollution. Both developed and developing countries share this burden, while developed countries have done a better job of maintaining their environment due to increased awareness and tougher legislation. Despite the fact that pollution has received worldwide attention, the impact is still felt due to its severe long-term implications. Concerns regarding raw material shortages, rising pricing, and general pollution have been at the forefront in recent years. As a result, challenges including waste recovery and recycling, as well as the transformation of trash into valuable products, are becoming increasingly important. Environmental pollution, particularly heavy metal contamination, is a severe environmental issue in today's society, and numerous solutions are being developed to clean it up. Heavy metal contamination is one of the most serious issues. Environmental pollution caused by heavy metals is one of the most serious issues confronting Egyptians. Metals are naturally occurring trace elements in the environment, but their concentrations have risen due to industrial waste, geochemical structure, farming, and mining operations. Heavy metals such as (As, Cd, Cu, Pb, Ni, and Zn) are common pollutants that come from both natural and anthropogenic sources. surfactant use has increased 300% in the United States during the last 20 years. Surfactants are now generated in excess of 3 million tons per year around the world. The majority of these surfactants can be chemically obtained from petroleum.

Biosurfactants are a heterogeneous group of surface-active particles released extracellularly or adjacent to the cell membrane by various types of bacteria and fungi. Biosurfactants are naturally amphiphilic, containing hydrophilic and hydrophobic moieties that reduce surface and interfacial tension while being

biodegradable, specific, and low in toxicity. These speeds up properties like foaming, emulsification, dispersing, and detergency. Biosurfactants are classified into two types: high molecular weight that contain polysaccharides and proteins, and low molecular weight that contain glycolipids and lipopeptides. They are classified as anionic, non-ionic, or cationic biosurfactants based on their charges. This encourages microbial growth by increasing the availability of hydrophobic nutrients and converting them into innocuous by products. Because of the unique property of lowering surface tension, the area of contact of insoluble compounds increases, as does the mobility, bioavailability, and biodegradability of hydrophobic pollutants. Biosurfactants have proven to be extremely beneficial to their producing strains by increasing the bioavailability of hydrophobic contaminants, connecting to heavy metals, possessing antimicrobial activity, and controlling the attachment and detachment of microorganisms to and from surfaces.

In this study, researchers were carried out on the production of biosurfactant and the use of it in different fields by *Bacillus spp., pseudomonas spp., Microbacterium spp. and Acinetobacter spp* isolated from soil samples by using 5 % olive oil as a substrate. 80 strains with different colony morphologies were isolated from soil samples taken from different regions in the Arab Republic of Egypt, and 20 of the strains determined to be positive for production of biosurfactant and were kept as stock culture and 7 strains with the highest production of biosurfactant among these strains. These strains were determined Using 27f/ 519r universal primers, the identity of the bacterial isolates were determined through amplification and sequencing of its 16s rRNA gene. The 16sRNA gene sequence of the bacteria was aligned with known sequences using the BLAST algorithm in the NCBI database. The bacterium was identified as *B.velezensis*, *B.haynesii*, *B.halotolerans*, *P.reinekei*, *M.arborescens*, *P.veronii* and *A.lwoffii*.

As a result of the Oil spread technique analysis, the highest biosurfactant production of (*B.velezensis*) was found after 15 days of Incubation in which the oil-free clearing zone diameter was 5.8 cm but of (*B.halotolerans* and *B.haynesii*) the highest production was at 72 h of Incubation in which the oil-free clearing zone diameter was 5.0 and 4.6 cm respectively. On the other side the highest biosurfactant production of (*M.arborescens*, *A.lwoffii* and *P.veronii*) was found after 72 h of Incubation in which the oil-free clearing zone diameter was 2.1 cm, 2.7 cm and 2.2 cm respectively and after 15 days for *P.veronii* making 2.0 cm of oil free zone but the same for *P.reinekei* there is no change in the oil-free clearing zone diameter 4.0 cm (Table 4.4).

In order to maximize the biosurfactant production of the isolates , the best conditions were provided by optimizing the biosurfactant production environment .As a result of carbon source optimization, maximum biosurfactant production was achieved by adding 5% olive oil without additional carbon source in the biosurfactant production medium with *B.velezensis*,*B.halotolerans*,*B.haynesii* and *M.arborescens* in which the oil-free clearing zone diameter was 4.0 cm ,5.0 cm ,4.6 cm and 2.1 cm respectively .On the other side in production environments containing 5% sucrose maximum biosurfactant production was achieved with *P.reinekei* and *P.veronii* in which the oil-free clearing zone diameter was 5.7 cm and 1.4 cm respectively (Table 4.24).

The highest biosurfactant production of the isolates, was obtained from biosurfactant production medium adjusted to pH 8 with *B.halotolerans* and *M.arborescens* in which the oil-free clearing zone diameter was 2.5 cm and 2.2 cm respectively , in production environments adjusted to pH 7 The highest biosurfactant production was obtained with *A.lwoffii* and *P.veronii* in which the oil-free clearing zone diameter was 2.0 cm and 1.8 cm respectively, On the other side the highest biosurfactant production was obtained at pH 6 with *B.velezensis* and *P.reinekei* in which the oil-free clearing zone diameter was 1.65 cm and 2.5 cm respectively. In *B.haynesii* the biosurfactant production was the same at pH 7 and

pH 8 in which the oil-free clearing zone diameter was 1.1 cm In the two microorganisms. The results obtained showed that the tested bacteria carried out biosurfactant production in a wide pH range (acidic, neutral and alkaline) (Table 4.22).

The biosurfactant activity determined after 72 hours incubation at 30ºC, 37ºC, 45ºC, As a result of temperature optimization, it was observed that maximum activity was obtained when the isolates were incubated at 37ºC, in *B.velezensis* , *B.halotolerans*, *A.lwoffii*, *B.haynesii*, *P.reinekei* and *M.arborescens* the oil-free clearing zone diameter was 4.0 cm , 5.0 cm , 2.7 cm , 4.6 cm , 4.0 cm and 2.1 cm respectively at 37ºC incubation but in *P.veronii* The highest biosurfactant production was at 30ºC incubation with 1.7 cm of the oil-free clearing zone diameter. When the results obtained were evaluated, it was seen that the isolates carried out biosurfactant production at mesophilic temperatures and the biosurfactant production decreased gradually at temperatures above 40ºC (Table 4.23).

In the emulsification index measurement of the isolates, it was observed that xylene and sunflower oil hydrocarbons decreased the surface tension by forming emulsion layers, but there are small or no emulsion layers were observed in the analysis made with n-heptane. it was observed that with *B.velezensis* xylene (E 47.6%) , sunflower oil (E 35.7%) and n-heptane (E 4.8%), with *B.halotolerans* xylene (E 19.0%) , sunflower oil (E 28.6%) and n-heptane (E 5.0%), with *B.haynesii* xylene (E 33.3%) , sunflower oil (E 34.1%) and n-heptane (E 6.0%), with *P.reinekei* xylene (E 23.3%) , sunflower oil (E 38.1%) and n-heptane (E 2.4%), with *M.arborescens* xylene (E 12.2%) , sunflower oil (E 24.4%) and n-heptane (E 2.4%), with *P.veronii* xylene (E 61.0%) , sunflower oil (E 36.5%) and n-heptane (E 12.2%), with *A.lwoffii* xylene (E 21.4%) , sunflower oil (E 17.1%) and n-heptane (E 4.9%). It was observed that the emulsions formed with xylene and sunflower oil remained stable even after 1 week but the emulsions formed with

n-heptane didn't remain stable after 1 week Except *P.veronii*, *B.halotolerans* and *B.haynesii* (Table 4.5 – 4.20).

The surface tension of the cell free culture broth obtained after 24 h, 48 h, 72 h and 15 days of incubation was measured by using the Optical Contact Angle / Surface Tension Measurement Device (TDIC - LAUDA) and the Ring method in Cukurova University, Faculty of Science and Letters, Biology Department, Bacteriology Laboratory. It was observed that the surface tension decreased from 50 mN/m in zero hours of incubation to 36.1 mN/m after 72 hours of incubation with *B.velezensis*, to 39.8 mN/m with *B.halotolerans* , to 42.5 mN/m with *B.haynesii* , to 42.5 mN/m with *P.reinekei* , to 46.3 mN/m with *M.arborescens* , to 44.7 mN/m with *P.veronii* ,and to 43.8 mN/m with *A.lwoffii*. After 15 days of incubation The surface tension decreased more with *P.reinekei* , *M.arborescens* , *P.veronii* , and *A.lwoffii* but with *B.velezensis* , *B.halotolerans* , and *B.haynesii* The surface tension increased at small rates (Table 4.3).

When the antimicrobial and antifungal activity of the isolates against pathogenic bacteria and fungi were examined, it was determined that the biosurfactant had bacteriostatic and bactericidal effects and the Antibacterial activity of the biosurfactant of *B.velezensis* was mostly against *E.coli 2*, *S.aureus* (Clinical isolate) 1,*S.aureus* (nature), *S.aureus* (Clinical isolate) 2,*Salmonella spp*., *E.coli 1* and *Klebsiella spp*., Although the Antibacterial activity did not occur against *S.aureus ATCC 29213*, the Antibacterial activity of *B.haynesii* was mostly against *E.coli 2*,*S.aureus* (Clinical isolate) 1, *S.aureus* (Clinical isolate) 2,*Salmonella spp*., *E.coli 1* and *Klebsiella spp*., but did not occur against *S.aureus ATCC 29213* and *S.aureus* (nature) , the Antibacterial activity of *B.halotolerans* was mostly against *E.coli 2*,*S.aureus* (Clinical isolate) 1,*S.aureus* (nature), *S.aureus* (Clinical isolate) 2,*Salmonella spp*. And *Klebsiella spp*., but did not occur against *S.aureus ATCC 29213* and *E.coli 1* , the Antibacterial activity of *P.reinekei* was mostly against *E.coli 2*,*S.aureus* (Clinical isolate) 1,*S.aureus* (nature), *S.aureus* (Clinical isolate) 2,*Salmonella spp*., *E.coli 1* and *Klebsiella spp*., but did not occur

against *S.aureus ATCC 29213* , the Antibacterial activity of *M.arborescens* was mostly against all of the tested pathogens *E.coli 2*,*S.aureus* (Clinical isolate) 1,*S.aureus* (nature), *S.aureus* (Clinical isolate) 2,*Salmonella spp*., *E.coli 1 Klebsiella spp*. And *S.aureus ATCC 29213*. the Antibacterial activity of *P.veronii* was mostly against *E.coli 2*, *Salmonella spp*., *E.coli 1* and *Klebsiella spp*., but did not occur against *S.aureus ATCC 29213*, *S.aureus* (Clinical isolate) 1,*S.aureus* (nature) and *S.aureus* (Clinical isolate) 2, and the Antibacterial activity of *A.lwoffii* was mostly against *Salmonella spp*.and *Klebsiella spp*. , but did not occur against *S.aureus* ATCC 29213, *S.aureus* (Clinical isolate) 1,*S.aureus* (nature) ,*S.aureus* (Clinical isolate) 2, *E.coli 1* and *E.coli 2*. it was determined that the biosurfactant of *B.velezensis*, *B.haynesii*, *B.halotolerans*, *P.reinekei* and *M.arborescens* had antifungal activity against *C.albicans* and the most powerful is *M.arborescens* (Table 4.25 – 4.33) .

Pollution is regarded as one of the most critical issues confronting human communities worldwide, particularly in developing countries. Despite the fact that it is caused by man and his actions, it has negative consequences for human environments and resources. As a result, pollution and its consequences are seen as one of man's most heinous sins against himself. Pollutants can cause primary harm, such as a direct influence on the environment, or secondary damage, such as subtle disruptions in the delicate balance of the biological food chain that are only noticeable over lengthy periods of time. Concerns regarding raw material shortages, increasing prices, and general pollution are at the forefront of today's society. As a result, challenges including waste recovery and recycling, as well as the transformation of waste into precious materials, are becoming increasingly important. Environmental pollution, particularly heavy metal contamination, has been identified as a severe environmental issue in recent years, and numerous solutions for its removal from the environment have been discovered.

Biosurfactants benefit their producing strains by increasing the bioavailability of hydrophobic pollutants, binding to heavy metals, having

antimicrobial activity, and regulating microbe adhesion to and detachment from surfaces. Because of their simple chemical structure, higher stability, strong foaming capability, low toxicity, pH, environmental compatibility, and biodegradability, biosurfactants outperform commercial surfactants. Biosurfactants have particular functional groups that allow them to detoxify certain contaminants under high salinity, temperature, and pH conditions. Biosurfactants are useful in the cosmetic, pharmaceutical, oil, and food industries because of their low toxicity and environmentally benign nature. The use of biosurfactants for some purposes was discovered to be both an environmentally benign and a cost-effective alternative to traditional sophisticated clean up techniques. Biosurfactants are being evaluated as a possible choice for pollutant clean up in the environment due to their diversity. Various assessment approaches have gained tremendous relevance in recent years. Their economic evaluation is at the forefront. Environmentally friendly technologies are built on the recycling of waste materials. These chemicals are used not only as bio emulsifiers and biosurfactants but also as antibiotics and biocontrol agents. Glycolipids, lipopeptides or lipoproteins, and polymeric components make up the majority of microbial surfactants. As a result, biosurfactants and their prospective applications have received more attention. Surface activity, pH, temperature, and ionic strength tolerance, biodegradability, low toxicity, emulsifying and demulsifying ability, and antimicrobial activity are all distinctive characteristics of microbial surfactants.

REFERENCES

Abalos A, Pinazo A, Infante M, Casals M, Garcia F, Manresa A. 2001. Physicochemical and antimicrobial properties of new rhamnolipids produced by Pseudomonas aeruginosa AT10 from soybean oil refinery wastes. Langmuir. 17:1367–1371. doi: 10.1021/la0011735.

Abdel-Mawgoud, A.M., Lepine, F. , Deziel, E. 2010. Rhamnolipids: diversity of structures, microbial origins and roles. Appl Microbiol Biotechnol 86, 1323–1336.

Abu-Ruwaida A., Banat I., Haditirto S., Salem A., Kadri M., 1991. Isolation of biosurfactant-producing bacteria, product characterization, and evaluation. Eng. Life Sci.,11(4), 315-324.

Accorsini, F. R., Mutton, M. J. R., Lemos, E. G., & Benincasa, M . 2012. Biosurfactants production by yeasts using soybean oil and glycerol as low cost substrate. Brazilian Journal of Microbiology, 43(1), 116–125.

Ahmad Z., Arshad M., Asghar H.N., Sheikh M.A., Crowley D.E. 2016. Isolation, Screening and Functional Characterization of Biosurfactant Producing Bacteria Isolated from Crude Oil Contaminated Site. Int. J. Agric. Biol., 18(3).

Al-Bager. S.D 2005. Bacillus cereus – King Saud University, College of Pharmacy, Department of Pharmaceutics, Section of Microbiology.

Anandara.B , P. Thivakaran .2010. Isolation and production of Biosurfactant producing organism from oil spilled soil, Journal of Biological science and Technology volume 1, ISSN: 120-126.

Asçi, Y., Nurbas, M. , Açikel, Y.S. 2008. A comparative study for the sorption of Cd(II) by soils with different clay contents and mineralogy and the recovery of Cd(II) using rhamnolipid biosurfactant. Journal of Hazardous Materials, 154: 663–673.

Badran M, Foster P .1998 .Environmental quality of the Jordanian coastal waters of the Gulf of Aqaba, Red Sea. Aquatic Ecosystem Health &Management 1(1): 75-89.

Banat, I.M., Franzetti, I.A., Gandolfi, G., Bestetti, M.G., Martinotti, L., Fracchia, T.J., Smyth And Marchant, R. 2010. Microbial biosurfactants production, applications. Applied Microbiology Biotechnology, 87: 427–444.

Banat, I.M., Makkar R.S. , Cameotra, S.S.(2000. Potential Commercial Applications of Microbial Surfactants, Applied Microbiol Biotecnol. 53, 495-508.

Batool R., Ayub S., Akbar I. 2017. Isolation of biosurfactant producing bacteria from petroleum contaminated sites and their characterization. Soil Environm ,36(1), 1-10.

Bodour Aa, Maier Rm. 2002. Biosurfactants: types, screening methods and application. In: Encyclopedia of environmental microbiology, 2nd edn, vol 2. Wiley, NY, p 750

Cameotra, S.S. , Makkar, R.S. 1998. Synthesis of biosurfactants in extreme conditions, Applied Microbiology and Biotechnology, 50: 520–529.

Cameotra, S.S. , R.S. Makkar, 2004. Recent applications of biosurfactants as biological and immunological molecules. Curr. Opin. Microbiol., 7: 262-266.

Chakrabarti, S. 2012. Bacterial biosurfactant: Characterization, antimicrobial and metal remediation properties. Ph.D. Thesis, National Institute of Technology.

Chrzanowski, Ł., Ławniczak, Ł. And Czaczyk, K. 2012. Why do microorganisms produce rhamnolipids? World J Microbiol Biotechnol 28, 401–419.

Cooper Dg, Goldenberg Bg .1987. Surface active agents from two Bacillus species. Appl. Environmen. Microbiol; 53(2):224 -229.

Cooper Dg, Macdonald Cr, Duff Sjb, Kosaric N. 1981. Enhanced production of surfactin from Bacillus subtilis by continuous product removal and metal cation additions, Appl. Environ. Microbiol; 42 : 408.

Das P, Mukherjee S, Sen R. 2008. Antimicrobial potential of a lipopeptide biosurfactant derived from a marine Bacillus circulans. J Appl Microbiol;104:1675–1684. doi: 10.1111/j.1365-2672.2007.03701.

Das, P., Mukherjee, S. And Sen, R. 2009. Antiadhesive action of a marine microbial surfactant. Colloids and Surfaces B: Biointerfaces, 71: 183–186.

Desai, J.D. And Banat, I.M. 1997. Microbial production of biosurfactants and their commercial potential. Microbiology and Molecular Biology Reviews, 61, 47-64.

Datta, P., P. Tiwari, And L. M. Pandey. 2018. "Isolation and characterization of biosurfactant producing and oil degrading Bacillus subtilis MG495086 from formation water of Assam oil reservoir and its suitability for en hanced oil recovery." Bioresour. Technol. 270 (Dec): 439–448.

Ebrahimi A., Tashi N., Lotfalian S. 2012. Isolation of biosurfactant producing bacteria from oily skin areas of small animals. Jundishapur J. Microb , 5(2), 401.

Ellis, W.D., Payne, J.R. And Mcnabb, G.D. 1985. Treatment of contaminated soils with aqueous surfactants, U.S. E PA 600/2–85/129.

El-Sheshtawy Hs, Aiad I, Osman Me . 2016. Production of biosurfactants by Bacillus licheniformis and *Candida albicans* for application in microbial enhanced oil recovery. Egyp J Pet.;25:293–298)).

Fernandes, P.A.V., Arruda I.R., Santos A.F., Araújo, A., Maior, M.A., Ximenes, E.A . 2007. Antimicrobial Activity of Surfactants Produced By Bacillus subtilis R14 Against Multidrug-Resistant Bacteria. Brazilian Journal of Microbiology, 38, 704-709.

Flasz, A., C.A. Rocha, B. Mosquera And C. Sajo . 1998. A Comparative study of the toxicity of a synthetic surfactant and one produced by Pseudomonas aeruginosa ATCC 55925. Med. Sci. Res., 26: 181-185.

Franzetti, A., Bestetti, G., Caredda, P., La Colla, P. And Tamburini, E. 2008. Surface–active compounds and their role in the access to hydrocarbons in Gordonia strains. FEMS Microbial. Ecology, 63: 238–248.

Garbarino Jr, Hayes H, Roth D, Antweider R, Brinton Ti, Taylor H .1995. Contaminants in the Mississippi River, U. S. Geological Survey Circular 1133, Virginia, U.S.A.

Gautam Kk, Tyagi Vk .2006. Microbial surfactants: A review. J Oleo Sci 55: 155-166.

Gomes, M.Z. And Nitschke, M. 2012. Evaluation of rhamnolipid and surfactin to reduce the adhesion and remove biofilms of individual and mixed cultures of food pathogenic bacteria. Food Control, 25, 441-447.

Hamed Ma, Mohamedein Li, El Sawy Ma, El Moselhy Km .2013. Mercury and tin contents in water and sediments along the Mediterranean shoreline of Egypt. The Egyptian Journal of Aquatic Research 39(2): 75-81.

Hashemi S.Z., Fooladi J., Ebrahimipour G., Khodayari S. 2016. Isolation and Identification of Crude Oil Degrading and Biosurfactant Producing Bacteria from the Oil-Contaminated Soils of Gachsaran. Appl. Food Biotechnol., 3(2), 83-89.

Hashim, M.A., Mukhopadhyay, S., Sahu, J.N. And Sengupta, B. 2011. Remediation technologies for heavy metal contaminated groundwater. Journal of Environmental Management, 92: 2355–2388.

Hawkins Jp, Roberts Cn .1994 .The growth of coastal tourism in the Red Sea: Present and future effects on coral reefs. Ambio 23(8): 503- 508.

Henkel, C.V. 2012. Primitive duplicate Hox clusters in the European eel's genome. PLoS One 7, e32231.

Herman, D.C., Artiola, J.F. , Miller, R.M. 1995. Removal of cadmium, lead and zinc from soil by a rhamnolipid biosurfactant. Environmental Science and Technology, 29: 2280–2285.

Hong, K.J., Tokunaga, S. , Kajiuchi, T. 2002. Evaluation of remediation process with plant–derived biosurfactant for recovery of heavy metals from contaminated soils. Chemosphere, 49: 379–387.

Hood Sk, Zottola Ea. 1995. Biofilms in food processing. Food Control 6: 9-18.

Igunnu E.T., Chen G.Z.2012. Produced water treatment technologies. LCT, 9(3), 157-177.

J.H. Unás, D. De Alexandria Santos, E.B. Azevedo, M. Nitschke. 2018. Brevibacterium luteolum biosurfactant: production and structural characterization, Biocatal. Agric. Biotechnol. 160–167.

Joshi, B.H.2010. Purification and Characterization of a Novel Protease from Bacillus firmus Tap5 Isolated from Tannery Waste. Journal of Applied Sciences Research, 6(8): 1068-1076.

Juwarkar, A.A., Nair, A., Dubey, K.V., Singh, S. And Devotta, S. 2007. Biosurfactant technology for remediation of cadmium and lead contaminated soils. Chemosphere, 68: 1996–2002.

Kahyaoglu, M. 1999. Production of Beta Carotene from Industrial Waste Materials by Microbial Way. Master Thesis, Anadolu University, Institute of Science and Technology, Eskisehir.

Karthik, L., Kumar, G., Rao, K.V.B. 2010. Comparison of methods and screning of biosurfactant producing marine actinobacteria isolated from nicobar marine sediment. The IIOAB Journal, Vol. 1; Issue 2, 34-38.

Kitamoto, D., Isoda, H. And Nakahara, T. 2002. Functions and potential applications of glycolipid biosurfactants: From energy–saving materials to gene delivery carriers. Journal of Bioscience and Bioengineering, 94: 187–201.

Kim Sy, Oh Dk, Lee Kh, Kim Jh .1997. Effect of soybean oil and glucose on sophorose lipid fermentation by Torulopsis bombicola in continuous culture. Applied Microbiol Biotechnol 48: 23-26.

Korayem As, Abdelhafez Aa, Zaki Mm . 2015. Optimization of biosurfactant production by Streptomyces isolated from Egyptian arid soil using Plackett–Burman design. Ann Agr Sci;60(2):209–217.

Mahalingam P., Sampath N. 2014. Isolation, characterization and identification of bacterial biosurfactant. Eur. J. Exp. Biol, 4(6), 59-64.

Maneerat, S. 2005. Production of biosurfactants using substrates from renewable-resources. Songklanakarin J. Sci. Technol., 27: 675-683.

Marchant, R. And Banat, I. 2012. Microbial biosurfactants: challenges and opportunities for future exploitation, Trends in Biotechnology 30: 558–565.

Margesin, R. And Shinner, F. 2001. Biodegradation and bioremediation of hydrocarbon in extreme environments. Applied Microbiology and Biotechnology, 56: 650–663.

Mccluggage D.1991. Heavy Metal Poisoning, NCS Magazine, Published by The Bird Hospital, CO, U.S.A. (www.cockatiels.org/articles/Diseases/metals.html).

Mcinerney, M.J., M. Javaheri , D.P. Nagle Jr. 1990. Properties of the biosurfactant produced by Bacillus licheniformis strain JF-2. J. Ind. Microbiol. Biotechnol., 5: 95-101.

Mohan, P.K., G. Nakhla , E.K. Yanful, 2006. Biokinetics of biodegradation of surfactants under aerobic, anoxic and anaerobic conditions. Water Res., 40: 533-540.

Morikawa M. 2000. BBA - Molecular and Cell Biology of Lipids, 1488:211. [PMID: 11082531].

Morrison, G.M.P. 1983. Urban storwater polluition: heavy metal studies of natural waters (a review). Research Report of Middlesex Polytechnic Research and Consultancy, Sweden.

Mulligan, C.N. 2005. Environmental Applications for Biosurfactants. Environmental Pollution, 133, 183-198.http://dx.doi.org/10.1016/j.envpol.2004.06.009.

Mulligan, C.N. 2009. Recent advances in the environmental applications of biosurfactants. Current Opinion in Colloid and Interface Science, 14: 372–378.

Mulligan, C.N. , Wang, S. 2004. Remediation of a heavy metal contaminated soil by a rhamnolipid foam. In: Geoenvironmental engineering, Integrated management of groundwater and contaminated land, London: Thomas Telford; pp. 544–551.

Mulligan, C.N., R.N. Yong , B.F. Gibbs. 2001. Remediation technologies for metal-contaminated soils and groundwater: An evaluation. Eng. Geol., 60: 193-207.

Nadeem, M., Qazi, J.I., Baig, S., Syed, Q.U.A.2007. Studies on commercially important alkaline protease from Bacillus Licheniformis N-2 isolated from decaying organic soil. Turkish Journal of Biochemistry, 32(4); 171-177.

Nash, J., Traver, R.P. , Downey, D.C. 1987. Surfactant enhanced in situ soil Washing. US.EPA HWERL. Edilsin, New Jersey.

Noudeh, G.D., Moshafi, M.H., Khazaeli, P., Akef, F. 2010. Studies on Bioemulsifier Production by Bacillus licheniformis PTCC 1595. African Journal of Biotechnology, Vol. 9(3), pp. 352-356.

Ochoa–Loza, F.J., Noordman, W.H., Jannsen, D.B., Brusseau, M.L. , Maier, R.M. 2007. Effect of clays, metal oxides, and organic matter on rhaminolipid biosurfactant sorpition by soil. Chemosphere, 66: 1634–1642.

Olkowska, E., Polkowska, Z. , Namiésnik, J. 2012. Analytical procedures for the determination of surfactants in environmental samples. Talanta, 88: 1–13.

Omar Hedm .2013. Seasonal variation of heavy metals accumulation in muscles of the African Catfish Clarias gariepinus and in River Nile water and sediments at Assiut Governorate, Egypt. Journal of Biology and Earth Sciences 3: 236-248.

Pacwa-Płociniczak M., Płaza G.A., Piotrowska-Seget Z., Cameotra S.S. 2011. Environmental applications of biosurfactants: recent advances. Int. J. Mol. Sci., 12(1), 633-654.

Pantazaki A.A., Papaneophytou C.P., Lambropoulou D.A. 2011. Simultaneous polyhydroxyalkanoates and rhamnolipids production by Thermus thermophilus HB8. AMB Express, 1(1), 17.

Parthipan P. Frontiers in microbiology. 2017. 8:193. [PMID: 28232826].

Pattanathu K.S.M. Rahman , Edward Gakpe, 2008. Production, Characterisation and Applications of Biosurfactants-Review. Biotechnology, 7: 360-370.

Peng, J.F., Song, Y.H., Yuan, P., Cui, X.Y. , Qiu, G.L. 2008. The remediation of heavy metals contaminated sediment. Journal of Hazardous Materials, 161: 633–640.

Peplow D .1999. Environmental Impacts of Mining in Eastern Washington, Center for Water and Watershed Studies Fact Sheet, University of Washington, Seattle.

Pereira J, Gudia E, Dria M, Domingues M, Rodrigues L, Teoxeira J, Coutinho J .2012. Characterization by electrospray ionization and tandem mass spectrometry of rhamnolipids produced by two Pseudomonas aeruginosa strains isolated from Brazilian crude oil. Eur J Mass Spectrom 18:399.

Poremba, K., W. Gunkel, S. Lang , F. Wagner. 1991. Toxicity testing of synthetic and biogenic surfactants on marine microorganisms. Environ. Toxicol. Water Qual., 6: 157-163.

Rita De Cássia F. S. Silva, Darne G. Almeida, Raquel D. Rufino, Juliana M. Luna, Valdemir A. Santos , Leonie Asfora Sarubbo .2014 Applications of Biosurfactants in petroleum industry and the remediation of oil spills. International journal of molecular science- ISSN 1422-0067.

Ron, E.Z. , E. Rosenberg. 2001. Natural roles of biosurfactants. Environ. Microbiol., 3: 229-236.

SANG Y, BLECHA F. 2008. Antimicrobial peptides and bacteriocins: alternatives to traditional antibiotics. Anim Health Res Rev.9:227–235. doi: 10.1017/S1466252308001497.

Sharma D, Mandal Sm, Manhas Rk. 2014. Purification and characterization of a novel lipopeptide from Streptomyces amritsarensis sp. nov. active against methicillin-resistant *Staphylococcus aureus*. AMB Express.4:1–9. doi: 10.1186/2191-0855-4-1.

Sidal, U. 1997. b sp. Obtaining Biosurfactant from Olive Oil Factory Waste with Doctoral Thesis, Trakya Institute, Edirne.

Singh, A., Van Hamme, J.D. , Ward, O.P. 2007. Surfactants in microbiology and biotechnology: Part 2, Application Aspects. Biotechnology, 25: 99–121.

Singh P, Cameotra Ss. 2004. Enhancement of metal bioremediation by use of microbial surfactants. Biochem Biophys Res Commun 319:291–297.

Sneha-Chakrabarti .2013. Bacterial Biosurfactant: Characterization, Antimicrobial and metal remediation properties.

Tabatabaee A., Assadi M.M., Noohi A., Sajadian V. 2005. Isolation of biosurfactant producing bacteria from oil reservoirs. J. Environ. Health Sci., 2(1), 6-12.

Tan, H.M., Champion, J.T., Artiola, J.F., Brusseau, M.L. , Miller, R.M. 1994. Complexation of cadmium by a rhamnolipid biosurfactant. Environmental Science and Technology, 28: 2402–2406.

Uyar Ve Yalçin, H.T. 2013. Mikrobiyal Biyosürfaktanlar. Türk Bilimsel Derlemeler Dergisi 6 (2): 93-102.

Varjani. J.S, D. P. Rana, S. Bateja, M. C. Sharma , V. N. Upasani .2014. Screening and identification of biosurfactant (bioemulsifier) producing bacteria from crude oil contaminated sites of Gujarat, India. International Journal of Innovative Research in Science, Engineering and Technology – Vol. 3, Issue 2.

Velikonja, J. , N. Kosaric .1993. Biosurfactants in Food Applications. In: Biosurfactants: Production: Properties: Applications, Kosaric, N. and F.V. Sukan (Eds.). Chapter 16, CRC Press, New York, ISBN-13: 9780824788117, pp: 419-448.

Vijayakumar S., Saravanan V. 2015. Biosurfactants-types, sources and applications. Res. J. Microbiol., 10(5), 181-192.

V.M. Alvarez, D. Jurelevicius, J.M. Marques, P.M. De Souza, L.V. De Araújo, T.G. Barros, R.O.M.A. De Souza, D.M.G. Freire, L. Seldin. 2015. Bacillus amyloliquefaciens TSBSO 3.8, a biosurfactant-producing strain with biotechnological potential for microbial enhanced oil recovery, J. Colloids Surf. B 136 14–21.

Walker Di, Ormond Rfg .1982. Coral death from sewage and phosphate pollution at Aqaba, Red Sea', Marine Pollution Bulletin 13(1): 21-25.

Wang, J., Ma, T., Zhao, L., Lv, J., Li, G., Zhang, H., Zhao, B., Liang, F. And Liu, R. 2008. Monitoring exogenous and indigenous bacteria by PCRDGGE technology during the process of microbial enhanced oil recovery. Journal of Industrial Microbiology and Biotechnology, 35: 619–628.

Wen, J., Stacey, S.P, Mclaughlin, M.J. And Kirby, J.K. 2009. Biodegradation of rhamnolipid, EDTA and citric acid in cadmium and zinc contaminated soils. Soil Biology Biochemistry, 41: 2214–2221.

Yadav A.K., Manna S., Pandiyan K., Singh A., Kumar M., Chakdar H.. 2016. solation and characterization of biosurfactant producing Bacillus sp. from diesel fuel-contaminated site. Microbiology, 85(1), 56-62.

Yount Ny, Yeaman Mr. 2013. Peptide antimicrobials: cell wall as a bacterial target. Ann N Y Acad Sci;1277:127–138. doi: 10.1111/nyas.12005.

Youssef Dh, El Said Gf .2011. Assessment of some heavy metals in surface sediments of the Aqaba Gulf, Egypt. Environ Monit Assess 180(1-4): 229-242.

Zaki Ms, Authman Mm, Hammam Amm, Shalaby Si .2014. Aquatic Environmental Pollution in the Egyptian Countryside and Its Effect on Fish Production (Review). Life Science Journal 11(9): 1024-1029.

RESUME

She completed her primary, secondary and high school education in Aswan. In 2012, she started her undergraduate education at Biology Department, Faculty of Science, Aswan University. She graduated with a Bachelor of Chemistry and Microbiology in 2015. In 2018, she started her Master's education in the Biology Department of the Institute Of Natural And Applied Sciences at Cukurova University and She is still continuing her education.