SCREENING OF BIOSURFACTANT PRODUCTION BY BACTERIAL STRAINS ISOLATED FROM OIL CONTAMINATED SITES NEAR GAS STATIONS IN EGYPT.

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ABSTRACT

Biosurfactant producing bacteria (28 isolates) were isolated from 30 oil contaminated soils and tested for production of biosurfactant by different screening methods. About 75% of the isolated bacteria showed no blood hemolysis and their emulsification index for hexane and xylene ranges from 28-35% and 30-45% respectively. Biosurfactant producing ability was confirmed by other tests where about 40% of isolates showed positive oil spreading activity, hydrocarbon overlay and 67% showed to be positive for drop collapsing test. 16s RNA sequencing of the most active isolates revealed one *Stenotrophomonas maltophila,* two *Bacillus spp.* and two *Achromobacter spp.* Biosurfactants extracted from these isolates showed variable antimicrobial activity against *Staphylococcus aureus, E. coli* and *Pseudomonas aeruginosa*. This study demonstrated that the biosurfactants produced by these bacteria could be used in combination with antibiotics for treating bacterial infections. Further study is required for enhancing biosurfactant production by bacterial isolates to be used environmentally for bioremediation of oil contaminated soils.

Keywords: biosurfactants, emulsification index, drop collapsing, *bacillus* spp.

{Mulligan, 1984 #26}{Techaoei, 2007 #13@@author-year}Introduction:

Surface active agents (surfactants) are amphiphilic molecules having the ability to reduce the surface and interfacial tension (Sridhar *et al*., 2015) Surfactants represent an important class of chemical products owing to their surface active properties showing industrial, agricultural and environmental applications (Nitschke & Costa, 2007). On the other hand these synthetic compounds are toxic and hard to be biodegraded representing a threatening source of environmental pollution (Tabatabaee *et al*., 2005; Saravanan & Vijayakumar, 2012). Recently, a great attention was given to biologically produced surfactants (biosurfactants or bioemulsifiers) which are diverse group of compounds produced by microorganisms(Sridhar *et al* .,2015).

Biosurfactants are considered a valuable gift to nature due to their safety, production on a large scale, selectivity and their efficiency under extreme environmental conditions(Siddiqui *et al*., 2015). In addition, lower toxicity, higher biodegradability(Shekhar *et al*., 2015) and being produced from cheap and renewable resources(Liu *et al*., 2013) increase their value. The production of different biosurfactants classes such as glycolipids, lipopeptides, phospholipids, neutral lipids or fatty acids and polymeric biosurfactants by different microorganisms was

reported by (El-Sheshtawy & Doheim, 2014). Contamination of terrestrial and aquatic ecosystems with oil results in emergence of microbial community capable of biosurfactant production and it greatly opens promosing ways for isolation of novel bioemulsifier producing strains(Chikere *et al*., 2009; Panjiar *et al*., 2015) *Acinetobacter, Pseudomonas, Bacillus, Rhodococcus, Acinetobcter* and *Enterobacter* have been reported to be biosurfactent producers(Panjiar *et al*., 2015).

Variable applications of biosurfactants result from their functional diversity(Shekhar *et al*., 2015) ,where these molecules could be beneficial in many different fields such as heavy metals, pesticides and hydrocarbon bioremediation in sites contaminated with them(Juwarkar & Yadav, 2008), also variable applications in food, cosmetic and pharmaceutical industries(Thanomsub *et al*., 2004).

The incredible interest given to the naturally produced amphiphilic biosurfactants (BS) and bioemulsifiers (BE) compounds is due to potential use instead of synthetic surfactants and so they may be applied in industry and environment (Satpute *et al*., 2010).

The objective of this study was to isolate bacteria from oil contaminated soils and screening of their biosurfactant producing ability. As well as studying the biosurfactant which are extracted from different isolates then screening of its antimicrobial activity against some bacteria was carried out.

Materials and Methods:

-Collection of oil contaminated soil samples:

Soil samples were collected from areas near different gas stations in 6 th October city and Giza in Egypt starting from 15th June,2016 till 20th March, 2017. The samples were collected in sterile polyethylene bags and taken directly to the laboratory for analysis. {SUDHANSHU SHEKHAR, 2015 #18}{Bodour, 1998 #11}

-Isolation of bacterial isolates by the enrichment culture technique:

One gram of each soil sample was inoculated into 50 mL of minimal salt medium containing (g/L); 15 g NaNO3, 1.1 g KCl, 1.1 g NaCl, 0.00028 g FeSO4.7H2O, 3.4 g KH2PO4, 4.4 g K2HPO4, 0.5 g MgSO4.7H2O, 0.5 g yeast extract at 37°C in shaker incubator (100 rpm). After incubation for 48h, samples were serially diluted utilizing sterile saline (0.85% NaCl) and different bacterial isolates were selected based on the colony morphology tryptic soya agar. Some isolates were screened for biosurfactant production using different screening methods. Modifications "subculture for 3 days on fresh MSM containing 1% oil.{Anandaraj, 2010 #6}{Tabatabaee, 2005 #2}{Carrillo, 1996 #12}{Abu-Ruwaida, 1991 #9}

-Screening methods for biosurfactant production by the isolated microorganisms:

-Blood hemolysis test:

Freshly prepared blood agar was inoculated with pure culture of bacterial isolates and incubated at 37°C for 48-72 h. According to the clear zone observed, results were determined where, α-hemolysis when the colony was surrounded by greenish zone, β-hemolysis when the colony was surrounded by a clear white zone and γ-hemolysis when there was no change in the medium surrounding the colony(Siddiqui *et al*., 2015).

-Emulsification index test (E_{24}):

In test tubes containing 2 mL of MSM, several colonies of pure cultures were suspended and incubated for 48 h then 2 mL hydrocarbon (oil) was added to each tube. Then, the mixture was vortexed at high speed for 1 min and allowed to stand for 24 h. The emulsification index (E24) is calculated as follow (Bodour *et al*., 2004)

$$\text{Emulsification index (E24)} = \frac{\text{Height of the emulsion layer}}{\text{Total height}} \times 100$$

-Oil-spreading test:

Oil spreading experiment was performed according to the method described by (Morikawa *et al*.., 2000). To a plastic petri dish, 20 ml of distilled water was added then 20 µl of crude oil was added to the surface of the water. Cell free culture broth (10 µl) was then added to the oil surface. According to presence or absence of biosurfactants in the cell free culture broth, displacemen of oil with an oil free clearing zone occurs and diameter of this clearing zone indicates the surfactant activity. A negative control (distilled water without surfactants) where no oil displacement was maintained.

- Drop collapsing test:

Biosurfactant production was also performed using the qualitative drop-collapse test. To the well regions delimited on the covers of 96-well micro plates, 2 µl of oil was applied, left to equilibrate for 24 h. From 48 h culture, 5 µl was centrifuged at 12000 g for 5 mins,translocated to the oil-coated well regions then observing drop size after 1 min, Positive biosurfactant production was documented when the drop was flat while cultures giving rounded drops were negative lacking biosurfactant production.

-Hydrocarbon overlay agar method:

Crude oil-coated LB agar plates were inoculated with overnight growth culture of the tested isolates, incubated at 30°C for 48 h. Emulsified halos around colonies indicate positive test for biosurfactant production (Siddiqui *et al*., 2015).

-Genotypic characterization of the isolates with biosurfactant producing activity by 16S rRNA gene sequencing:

- DNA isolation and manipulation:

DNA isolation from isolated bacteria was carried out by boiling method according to Dashti *et al*.,(2014) with some modifications. One ml of overnight growth bacterial culture in ependorff tube was centrifuged at 6000rpm for 10 min, discard the supernatant and 200µl of sterile distilled water was added to the pellets and heated in water bath at 95°C for 30 mins. The tube was vortexed and centrifuged at 6000 rpm for 15 mins, the supernatant "contain isolated DNA" was carefully transferred to another clean tube. For purification of DNA extract, the same volume of isopropanol was added to the supernatant for 10 mins and centrifuged at 13000 rpm for 20 mins. The supernatant was discarded and pellets were washed with 70% ethanol for

10 mins and centrifuged. To the obtained washed pellets, 50µl of sterile distilled water was added "this is DNA ready for PCR.

Identification of biosurfactant producing isolates by sequencing of partially amplified 16S rRNA gene of the bacterial isolates:

Biosurfactant producing isolates (E1,E2,E3,E6 and E7) were further identified by 16S rRNA gene sequencing. For these isolates, the 16S rRNA gene was amplified using universal primers pair:

1F (5-GAGTTTGATCCTGGCTCAG-3) and 6R (5-AGAAAGGAGGTGATCCAGCC-3) recorded to produce an amplicon of approximately 1500 base pair (bp).

Primers solutions were prepared according to the instructions of the manufacturer to a concentration of 100 µM stock solution using nuclease free water.

PCR was carried out in a total volume of 50 µl using 25 µl of MyTaq PCR Master Mix (2X) (Bioline, Germany), containing 1 µl (10 µM) of each forward and reverse primers together with equivalent microliters to 50 ng of the genomic DNA and finally completed to 50 µl by nuclease free water. Thermo cycling was carried out using thermo cycler (Biometra, Germany). The amplification was verified by gel electrophoresis using 1% (w/v) agarose gel supplemented with 0.2 µg/ml of ethidium bromide.

PCR products were purified by Gene Jet Gel Extraction Kit (Thermo Fisher Scientific, USA) according to manufacturer protocol. The purified products were sent for sequencing at Macrogen, Korea. Nucleotide sequence similarities were determined using other known sequences found in the GenBank database using BLAST program of National Center for Biotechnology Information (NCBI) databases.

Extraction and purification of biosurfactant from the bacterial isolates:

The samples of the selected cultures were centrifuged at 10,000 rpm for 15 min to remove the bacterial cells. 6 M HCl was added to the supernatant to reach pH 2.0. Surfactin precipitation was formed by settling overnight at 4C°. The precipitate was collected by centrifugation at 10,000 rpm for 15 min at 45 °C to obtain the crude surfactin. The crude extract was dissolved in deionized water (pH 8.0) to a finally pH 7 and extracted by 65:15 (v/v) (chloroform/ethanol) at room temperature. The solvent was evaporated by a rotary evaporator, yielding pure biosurfactant product.

Antimicrobial activity of biosurfactants:

The five extracted biosurfactants of the selected 5 isolates (E1, E2, E3, E6 and E7) were tested for its antimicrobial potential by agar diffusion "cup plate technique" against different isolates "*E coli, Pseudomonas aeruginosa* and *Staphylococcus aureus".* A total of 15 Muller-Hinton plates and 5 plates were swabbed with *E. coli*, 5 with *Pseudomonas aeruginosa* and *another* 5 with 5 with *Staphylcoccus aureus.* On each plate, 3 wells were made designated as X,Y&Z referring to the extracted biosurfactantat, diluted 10 fold biosurfactants and sterile distilled water. The 15 plates were 5 plates were swabbed with *E. coli.* The antimicrobial activity of each biosurfactant was tested against each isolate at the main concentration and 10 fold diluted concentration using water as a negative control.

The plates were incubated at 37°C for 24 hours. The presence of clear zone marked the antimicrobial activity of biosurfactant. Three readings of the clear zone diameter were taken for each well and the mean was calculated to determine the actual zone diameter (Rodrigues *et al.*, 2006).

Results:

A total of 28 isolates were isolated from different 30 oil contaminated soil samples by enrichment culture technique. Pure colonies isolated on MSM agar plates were further screened for biosurfactant producing activity by hemolytic activity, oil spreading test, emulsification index test hydrocarbon overlay agar methods.

The results showed that only 10% were positive for blood hemolysis "B-hemolysis", 15% showed partial hemolysis and 75 % showed no hemolysis.

Upon testing the emulsification index test (E24%) of both hexane and xylene for the 28 isolates, the results are shown in table 1.

Of the total number of tested bacterial isolates, 55% and 58 % showed 35-45% E24 for hexane and xylene respectively. Also E 24 % of 46-55 for hexane and xylene were 28% and 30% of the tested isolates respectively.

The highest % of E 24 (56-65%) for hexane and xylene were obtained by 17 % and 12% of the total tested isolates respectively.

Table 1: Emulsification index for hexane and xylene of the isolated bacteria

Emulsification index %	**%No of isolates**	
	Hexane	**Xylene**
35-45	**55%**	**58%**
46-55	**28%**	**30%**
56-65	**17%**	**12%**

By testing the oil spreading activity of the 28 isolates, 40% were positive for oil spreading activity (where 18% and 22% showed the highest and lowest activity respectively). About 60% of isolates showed no activity at all (Results are shown in fig 1).

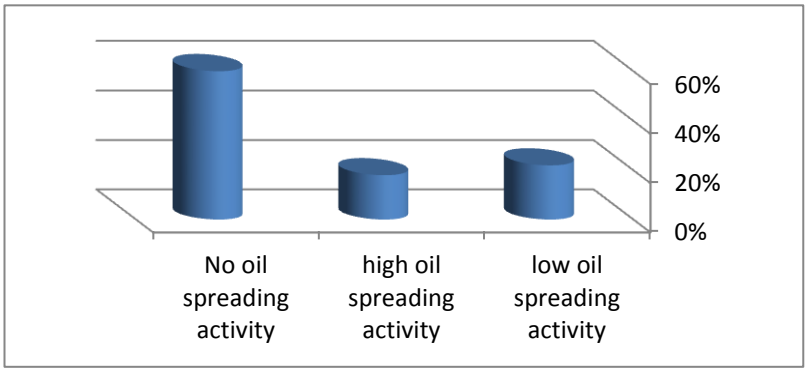

Figure 1: oil spreading activity of the tested isolated bacteria.

Drop collapsing method for the isolated bacteria revealed the biosurfactant producing activity of the isolated bacteria where the flat drop appearance in microtiter plate by some isolates confirmed positive test.

Among the 28 strains screened, 16 (42.8 %) strains were positive for drop collapse activity.

Hydrocarbon overlay agar test was also carried out for the isolated bacteria and the results showed that nearly all isolates were positive for hydrocarbon overlay agar where about 40 % of the tested bacteria for hydrocarbon overlay agar method showed to be positive for the emulsified halos around the colony.

Genotypic characterization of the most significant bioemulsifier-producing microorganisms was done by 16s RNA identification. The sequences were compared to GenBank sequence database of NCBI for analysis using the online program http://blast.ncbi.nlm.nih.gov/Blast.cgi. Sequence blast showed that the isolate No. (E1) had a high similarity 89 % to *Stenotrophomonas maltophilia strain LMG 11114 (*X95925.1) and 88 % similarity to *Stenotrophomonas sp. Toyama-1*(AB180662.1). While isolate (E2) had a high similarity 99 % to *Bacillus aerophilus strain DHN10.4*(KX809598.1) and 98% similarity to *Bacillus xiamenensis strain MCCC 1A00008* (NR_148244.1). The isolate (E3) showed 86% similarity to *Bacillus subtilis* subsp. inaquosorum strain BGSC 3A28(NR_104873.1) and 86% similarity to *Bacillus subtilis* subsp. spizizenii strain NBRC 101239 (NR_112686.1).For isolate (E6) it had a high similarity of 98 % to *Achromobacter pulmonis* strain NPU (MH595937.1) and 98% to *Achromobacter xylosoxidans* strain SOLR10 (CP025774.1). Also, isolate (E7), showed a similarity of 100% to *Achromobacter sp*. strain YJN-1(MF993519.1) and 100% similarity to *Achromobacter pulmonis* strain BFHA4 2(MG897140.1).

The extracted biosurfactants from the above identified five isolates were screened for their antimicrobial activity against both gram negative bacteria "*E. coli* and *Pseudomonas aeruginosa*" and gram positive "*Staphylococcus aureus*" by cup plate technique. The inhibition zones of bacterial growth of the tested bacteria upon addition of the five tested biosurfactants were determined and the results are represented in table 2.

Table 2: Antimicrobial activity of the extracted biosurfactants

Tested biosurfactant	**Zone of inhibition diameter(mm)**								
	Pseudomonas aeruginosa			***E. coli***			***Staphylococcus aureus***		
	X	**Y**	**Z(control)**	**X**	**Y**	**Z(control)**	**X**	**Y**	**Z(control)**
E1	**1.3**	**1.1**	**-**	**1.9**	**1.6**	**-**	**3**	**2.8**	**-**
E2	**1.2**	**1**	**-**	**2.4**	**2.2**	**-**	**3.7**	**3.5**	**-**
E3	**1.4**	**1.2**	**-**	**2.8**	**2.3**	**-**	**3.9**	**3.6**	**-**
E6	**1.8**	**1.2**	**-**	**3**	**2.5**	**-**	**3.8**	**3.7**	**-**

Discussion:

Due to the current antibiotic resistance problem worldwide, microbial bio surfactants are currently investigated as an alternative antimicrobial tool to other potential antimicrobial sources (Banat, 2000).

In the present study, 30 oil contaminated soil samples were collected from different areas near gas stations, sub-cultured on MSM containing oil. A total of 28 bacterial isolates were recovered, purified and screened for their bio surfactant producing ability by different tests

including hemolytic activity, emulsification index test, oil spreading test and hydrocarbon overlay agar.

As previously reported by (Satpute *et al*., 2010), several screening methods could be included in the primary screening of potential biosurfactant production. Where a single method isn't suitable for determination of biosurfactant production from isolated bacteria (Satpute *et al*., 2008).

The present study showed that about 10% of isolated bacteria showed B-hemolysis, 15% partially hemolysed blood and 75% showed no hemolysis. (Carrillo *et al*.,1996) found an association between hemolytic activity and biosurfactant production and they recommended the use of blood agar lysis as a primary method for biosurfactant production screening. On the other hand, strains with positive hemolytic activity were found to be negative for biosurfactant production (Youssef *et al*., 2004 and Thavasi *et al.,* 2011). In addition, not all biosurfactants have hemolytic activity and compounds other than biosurfactants may cause hemolysis. The possibility of biosurfactant production without a hemolytic activity wasn't previously reported (Satpute *et al*., 2010 and Walter *et al*., 2010).

Emulsification activity of the isolated bacteria was tested by calculation of emulsification index where the emulsifying ability of culture supernatant could be determined by the emulsification index (E-24) test (Panjiar *et al*.,2015).

We declared that 58% and 55% of the isolates showed 30-45% E24 for xylene and hexane respectively while low percentages of isolates 17% and 12% showed E24% of 56-65% for hexane and xylene respectively. In a previous study by (Patowary *et al*., 2017), it was reported that the biosurfactant maximum emulsification activity was 100% for crude oil, followed by diesel, kerosene and engine oil. Bento *et al*., (2005) isolated four *Bacillus* species and one *Acinetobacter* which showed an obvious ability to decrease surface tension and increase emulsification activity.

A similar previous study by (Bonilla *et al*., 2005) reported that the emulsifying activities (E24) determine the bioemulsifier productivity and are given as a percentage of the height of the emulsified layer divided by the total height of the liquid column. Also, it was investigated in another study that 6% of the total isolates produced negative emulsification potential, and almost 33% gave a good emulsification index with tested hexane, xylene, and crude oil. Ability of bacteria to adhere to hydrocarbons is a characteristic feature of biosurfactant-producing capability of microorganisms (Siddiqui *et al*., 2015).

Bacillus licheniformisis showed the ability to produce biosurfactant which siginificantly lowered the surface tension of various liquids (Karlapudi *et al*., 2018)

A *Serratia marcescens* BS-03 was isolated from oil contaminated soil among other 42 isolates, and showed the highest emulsification activity with the highest emulsification index (Budsabun, 2015). The ability of oil spreading activity of the isolated microorganisms was tested and it was found that about 42% of the isolated bacteria showed positive oil spreading activity while 18% of them were high spreading activity. A study by Panjiar *et al*.,(2015) indicated that oil spread test performed with diesel sorted all the screened micro-organisms into two major groups: A (diameter of clear zone in the range of 0–4.9 cm) and B (diameter of clear zone ≥5 cm) with 63 and 25 microorganisms, respectively, in each group.

Another method revealing the biosurfactant producing activity of the isolated bacteria is drop collapsing test, where the flat drop appearance in microtiter plate by some isolates

confirmed positive test. Drop-collapse assay technique relies on the destabilization of liquid droplets by surfactants(Jain *et al*., 1991).

The findings of the present study revealed that 12 bacterial isolates (out of 28) had a positive drop collapse (qualitative) test. This may be an evidence that that the isolates are able to produce surface active compounds, which caused reduction in surface tension. Satpute *et al*., (2010) reported that the drop collapse and oil spread tests can be used together for primary screening of biosurfactant-producing isolates due to their high sensitivity.

For further confirmation of the emulsification ability of the isolated bacteria, hydrocarbon overlay agar method was carried out and it showed that 40% of all screened isolates gave positive results. A previous study by (Siddiqui *et al*., 2015) showed halos around the colony for 65.1% of all tested isolates. The results of drop collapsing test indicated that nearly all isolates positive for hydrocarbon overlay agar method also showed to give positive drop collapse test.

The most active five biosurfactant producing bacterial isolates were further identified by 16S rRNA gene sequencing. Upon comparing the sequences of the tested isolates to GenBank sequence database, the results revealed that isolate No (E1) had a similarity (89%) to *Stenotrophomonas maltophilia* strain. While isolate (E2) had a high similarity (99%) to *Bacillus aerophilus* strain DHN10.4 (KX809598.1) and 98% similarity to *Bacillus xiamenensis* strain MCCC 1A00008 (NR_148244.1).

The isolate (E3) showed 86% similarity to *Bacillus subtilis* subsp. inaquosorum strain BGSC 3A28 (NR_104873.1) and 86% similarity to *Bacillus subtilis* subsp. spizizenii strain NBRC 101239 (NR_112686.1). For isolate (E6) it had a high similarity of 98 % to *Achromobacter pulmonis* strain NPU (MH595937.1) and 98% to *Achromobacter xylosoxidans* strain SOLR10 (CP025774.1). For isolate (E7), a similarity of 100% to *Achromobacter pulmonis* strain BFHA4 2(MG897140.1) was indicated.

It was reported by Segura-Carretero *et al*., (2016) that *Stenotrophomonas* genus, a closely related strain to (B-2 strain) which showed an emulsification ability was isolated from petrochemical production water.

A recent study by (Haleem *et al*., 2018) reported that *Stenotrophomonas maltophilia* strain 5DMD, displayed oil displacement assay = > 6 mm; surface tension = 33.9 and 34.4 m N m^{-1} and this proved the high effciciency of biosurfactants production.

Upon growth of *Achromobacter xylosoxidans* GSMSR13B on MSM containing 1 % (v/v) of glycerol and 1.5 g/L of NH4NO3 with a C/N ratio of 16:1 at pH 8.0, incubated at 37 ºC and shaken at 150 rpm for 5 days. The biosurfactant produced reduced surface tension to 37.9 dynes/cm, representing a 57 % reduction in surface tension with emulsification index (%EI 24) of 47.4 % (Reddy *et al*.,2018).

A previous study by (Deng *et al*., 2014) demonstrated that , *Achromobacter sp.* HZ01 showed the ability to degrade petroleum hydrocarbons in areas contaminated with them, also other strains (*Achromobacter xylosoxidans* and other *Achromobacter sp.*) similarly showed alkane degradation. to strain HZ01. Hong *et al*., (2017) isolated a marine bacterium *Achromobacter sp.* HZ01, which showed the ability to degrade hydrocarbons and produce biosurfactants.

Bacillus sp. is commonly known for their ability to produce several biosurfactants, also *Bacillus subtilis* were known to synthesize biosurfactants (Shekhar *et al*., 2015) also, were reported to be one of the most commonly studied industrial microorganisms (Bento *et al*., 2005). *Bacillus* spp. Showed to produce two surface active agents: one is a d-glucosamine polymer and the other is a mixture of saturated monoglycerides. Only *B. subtilis* and *Bacillus pumilus* have been reported to produce surfactin (of known commercial application) (Bento *et al*., 2005). The oil emulsification index of hydrocarbon degrading microorganisms was found to be in the range of 25 to 54%. Amongst the isolates obtained, the *bacillus cereus* culture expressed the better oil emulsification and degradation ability. So *bacillus cereus* strain could show valuable role in the bioremediation of oil pollution (Gupte & Sonawdekar, 2015).

There are many reports that support the efficiency of *Bacillus spp*. on biosurfactant production and thus they have been widely used for many applications such as in oil recovery screening methods and oil displacement method was considerably good (Morikawa *et al*.,2000).

In a study by (Parthipan *et al*., 2017), The emulsifying activity for the biosurfactant produced by *Bacillus subtilis* A1 was determined and confirmed by the oil displacement test. Where a clear zone of 2.4 cm was visualized upon addition of surfactant solution in the crude oil layer.

From the most actively isolated biosurfactants producing bacteria, the biosurfactants were extracted and precipitated by HCL and chloroform/ethanol method.

The antimicrobial activity of the extracted biosurfactants against *E. coli, Pseudomonas aeruginosa* (gram negative bacteria) and *Staphylococcus aureus* (gram positive bacteria) were investigated by cup-plate technique. The results showed us the variation of the antimicrobial effect of the biosurfactants towards gram positive and gram negative isolates where, the tested biosurfactants is more effective against *Staphylococcus aureus* than against *E. coli* and *Pseudomonas aeruginosa*. This could be illustrated by their unique outer membrane lipopolysaccharide (LPS). The LPS either acts as a barrier or provides protection to the inner sensitive membrane and cell wall from the toxic compounds A similar previous study by (Patowary *et al*., 2017) reported that *B. subtilis, S. aureus, K. pneumonia, and E. coli* were susceptible to the purified biosurfactant where it showed antibacterial properties that inhibited both Gram +ve and Gram –ve strains.

Screening of crude biosurfactant compounds for its in-vitro antibacterial activity was carried out in several previous studies where, (El-Sheshtawy & Doheim, 2014) reported a good antimicrobial activity of a biosurfactant producing *Pseudomonas aeruginosa* against all tested different species of bacteria and fungi strains and (Shekhar *et al*., 2015) reported the presence of some therapeutic applications of biosurfactants.

Antibiotic therapies is the main choice for treatment of most human bacterial infections, however, in recent years, a significant increase in the emergence of pathogenic resistant microorganisms to most available antimicrobials has been observed, including multi-drug resistant (MDR) pathogens, which has been associated with the misuse or abuse of antibiotics.Therefore, finding biosurfactant producing microorganisms may be promising for enhancing antibiotics effect in treating microbial infections and hence decreasing the microbial resistance to the antibiotics used.(Coates *et al*., 2011 and Gudiña *et al*., 2016)

Conflict of interest:

The author declares no conflict of interest.

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فحص إنتاج العوامل الحيوية البيولوجية بواسطة السلالات البكتيرية المعزولة من المواقع الملوثة بالزيت بالقرب من محطات الوقود في مصر

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الملخص :

تم عزل البكتيريا المنتجة للعوامل الحيوية (٢٨ عزلة) من ٣٠ تربة ملوثة بالزيت وتم اختبارها من أجل إنتاج المادة الفعالة من خلال طرق الفحص المختلفة حيث وجد ان مؤشر الاستحلاب للهكسين وزيلين يتراوح بين ٢٨-٣٥ ٪ و ٣٠-٤٥ ٪ على التوالي. تم تأكيد القدرة على إنتاج العوامل الحيوية من خلال اختبارات أخرى حيث أظهر حوالي ٤٠ ٪ من العزلات نشاطًا إيجابيًا لنشر الزيت وتراكب الهيدروكربونات .وكشف تسلسل الحمض النووي الريبي s١٦ من أكثر العزلات نشاطا وهم ستينوتروفوموناس مالتوفيلا ، واثنين من الباسيلاس واثنين من الاكروموباكتير. أظهرت العوامل الحيوية المستخرجة من هذه العزلات نشاطًا مضادًا للميكروبات ستافيلوكوكاس اوريوس،ايشيريشيا كولاى،سودوموناس ايروجينوزا. أثبتت هذه الدراسة أن العوامل الحيوية التي تنتجها هذه البكتيريا يمكن استخدامها في تركيبة مع المضادات الحيوية لعلاج الالتهابات البكتيرية. هناك حاجة إلى مزيد من الدراسة لتعزيز إنتاج المواد الفعالة بيولوجيا بواسطة عزلات البكتريا لاستخدامها بيئيًا في معالجة التربة الملوثة بالزيت.

الكلمات المفتاحية: عوامل حيوية ، مؤشر الاستحلاب الباسيلاس، تراكب الكربوهيدرات.