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Production, Characterization and Antimicrobial Activity of a Bioemulsifier Produced by *Acinetobacter baumanii* AC5 Utilizing Edible Oils

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Abstract: Biosurfactant or Bioemulsifier amphiphilic compounds are; produced by microorganisms as primary or secondary metabolites. The unique properties of biosurfactants mean that they have the potential to supplement, or even replace, chemical surfactants used in food, in the cosmetics and pharmaceutical industries; and in the environment. In the present study ten *Acinetobacter* sp. isolated from different sources were tested for their capability to produce bioemulsifiers in mineral salt medium with addition of 1% (v/v) edible oil as the sole source of carbon. Out of the ten *Acinetobacter* isolates tested, five showed lipase activity and produced bioemulsifiers exhibiting an emulsification index (EI24%) of 40 – 78%. The results revealed that the isolate *Acinetobacter baumanii* AC5, a gram negative, oxidase negative, aerobic and a diplococcoid rod bacterium was the best bioemulsifier producer. Optimization studies indicated that bioemulsifier production was associated with bacterial growth, and that the presence of inducer edible oils in the medium also enhanced bioemulsifier production. On the other hand, bioemulsifier production decreased when hydrocarbon (gasoline and diesel oil) were used. Crude bioemulsifier was recovered from the culture supernatant by a solvent system of chloroform: methanol (2:1 v/v), with the extraction producing 5.05 g/l of crude bioemulsifier. Partial purification and chemical analysis of the bioemulsifier revealed that it is a lipoglycan in nature with lipid content of 63%, carbohydrate 35% and a minor fraction of protein 2%. The crude bioemulsifier; showed strong antimicrobial and antifungal activity against tested pathogenic microorganisms.

Key words: *Acinetobacter*, Bioemulsifier, Lipases, Oils, Lipoglycan, Antimicrobial.

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Introduction

Acinetobacter spp. is widespread in nature; and can be obtained from water, soil, living organisms and even from human skin. They are gram-negative, oxidase-negative, non-motile; and strictly aerobic and appear as coccobacilli in pairs under the microscope. Species of *Acinetobacter* have been attracting increasing attention in respect to both environmental and biotechnological applications. Some strains of this genus can degrade and remove a wide range of organic and inorganic compounds; including pollutants such as biphenyl and chlorinated biphenyl, amino acids (analine), phenol, benzoate and; crude oil (1).

Acinetobacter strains are also well represented among fermentable bacteria used in the production of a number of extra- and- intracellular economic

products; such as lipases, proteases, cyanophycine, bioemulsifiers and several kinds of biopolymers (2).

Biosurfactants / and bioemulsifiers are amphiphilic, and formed by two parts, a polar (hydrophilic) moiety usually consisting mono-, oligo-, or polysaccharides, peptides or proteins and a non-polar (hydrophobic) group containing saturated, unsaturated and hydroxylated fatty acids or fatty alcohols (3).

The microbial surfactants known as biosurfactants are microbial compounds that exhibit a broad diversity of chemical structures such as glycolipids, lipopeptides and lipoproteins, lipopolysaccharides, phospholipids, fatty acids and polymeric lipids. It is therefore reasonable to expect biosurfactants to possess diverse properties and physiological functions; such as increasing the surface area and bioavailability of hydrophobic water-insoluble substrates, heavy metal binding, bacterial pathogenesis, and quorum sensing and biofilm formation. They are useful as antibacterial, antifungal and antiviral agents, and adhesive agents (3).

Many strains of *Acinetobacter* from mud, soil, marine, fresh water, etc. have been reported to produce bioemulsifiers. The majority of *Acinetobacter* strains, including both hospital and environmental isolates produce high-molecular-mass bioemulsifiers. The most intensively studied of these are the bioemulsans produced by *Acinetobacter calcoaceticus* RAG-1 and *A. calcoaceticus* BD4 (4). The RAG-1 emulsan is a complex of an anionic heteropolysaccharide and protein. Its surface activity is largely due to the presence of fatty acids, comprising 15% of the emulsan's dry weight, which are attached to the polysaccharide backbone via O-ester and N-acyl linkages. The protein component of the RAG-1 emulsan stimulates the emulsifying activity (5). *Acinetobacter radioresistens* KA53 produces alasan, a complex of an anionic polysaccharide containing covalently bound alanine (apoalasan) and three proteins. It is released into the extracellular fluid during the stationary phase of the life cycle of this bacterium (6). Extracellular membrane vesicles partition hydrocarbons to form a microemulsion, which plays an important role in the uptake of alkenes by microbial cells. Vesicles of *Acinetobacter* sp. strain HO1-N with a diameter of 20–50 nm are composed of protein, phospholipids and lipopolysaccharide (7).

The carbon source used in bacterial culture is very important in biosurfactant production. In general it can be divided into three categories: carbohydrate, hydrocarbon and vegetable oils. Plant-derived oils can act as effective and cheap raw materials for biosurfactant production. For example, rapeseed oil, Babassu oil and corn oil. Vegetable oils such as soybean oil have been used for the production of rhaminolipid, sophorolipid and mannosylerythritol lipid biosurfactants by various microorganisms (8).

There are few reports of bioemulsifier production by *Acinetobacter spp.*, however, and it is this gap that has prompted us to screen *Acinetobacter* isolates for bioemulsifier production. The present research was aimed to study bioemulsifier production by *Acinetobacter* isolates from different sources**,** their partial purification and characterization, with its application as antimicrobial activity.

Material and Methods

Source of Bacterial Isolates for Bioemulsifier Production

Ten *Acinetobacter* isolates from contaminated soil, polluted water, and food sources were used in the current study. Identification of the isolates up to genus level was carried out by morphological and biochemical tests and further confirmed by Vitek – 2 Compact Biomerieux (USA).

Source of Bacterial Isolates in Antimicrobial Activity Test

The following bacteria were used to determine the antibacterial activity of the bioemulsifier: *E. coli*, *Salmonella*, *Staphylococcus aureus and Pseudomonas aeruginosa* (isolated from a clinical sample and obtained from the Biotechnology Department, College of Science, and Baghdad University). The isolates were grown on nutrient agar and incubated at 37 ^{o}C for 18 h.

Source of Fungal Isolates in Antimicrobial Activity Test

The antifungal activity of the bioemulsifier was determined against: *Penicillium notatum* and *Fusarium oxisporium* (isolated from infected crops), obtained from the Biotechnology Department, College of Science, University of Baghdad). The isolates were grown on potato dextrose agar (PDA) and incubated at 25 ^{o}C for 72 h.

Screening of *Acinetobacter* Isolates

The production of bioemulsifier from a pure culture of *Acinetobacter* isolates was determined by: lipase production and emulsification activity (EA) and emulsification index (EI 24%) measurement. For lipase activity Luria Bertani agar plates supplemented with 1% of olive oil emulsion were prepared (the pH of the medium was adjusted to 7.0) spot inoculated with a fresh culture of *Acinetobacter* isolates and incubated at 30°C for 3 days. After incubation, the plates were examined for a clear zone of hydrolysis around the colony (9). Positive isolates were screened for emulsification activity.

Screening of *Acinetobacter* Isolates for Bioemulsifier Production

Out of ten isolates, five cultures of *Acinetobacter* (AC1, AC5, AC6, AC8, and AC9) showing lipase activity were further screened for bioemulsifier production. The isolate which showed maximum activity was used for further identification and bioemulsifier studies.
To determine the bioemulsifier production ability, *Acinetobacter* isolates were grown in nutrient broth for 16–18 h at 30 ˚C. This culture was used as the stock culture inoculums at the 1% (v/v) level. For bioemulsifier production and surface active properties of the bioemulsifier, a mineral salt medium (MSM) with the following composition (g/L) was used (10): K_2HPO_4 (1), KH_2PO_4 (1), $MgSO_4 \cdot 7H_2O$ (0.6), $FeSO_4 \cdot 7H_2O$ (0.01), NaCl (0.05), $CaCl_2$ (0.02), yeast extract (0.5) and 0.1 mL of trace element solution containing (g/L): 2.32 g $ZnSO_4 \cdot 7H2O$, 1.78 g $MnSO_4 \cdot 4H_2O$, 0.56 g H_3BO_3, 1.0 g $CuSO_4 \cdot 5H_2O$, 0.39 g $Na_2MoO_4 \cdot 2H_2O$, 0.42 g $CoCl_2 \cdot 6H_2O$, 1.0 g EDTA, 0.004 g $NiCl_2 \cdot 6H_2O$ and 0.66 g KI. The pH of the medium was adjusted to 7.0. Bioemulsifier production was carried

out in 50 ml of the above medium in a 250 ml Erlenmeyer flask containing 1 % (v/v) of olive oil and incubated with continuous shaking (120 rpm) for 72 h at 30 ^{o}C using a shaker incubator (Basal Switzerland). Bioemulsifier production was determined by measuring the Emulsification Index (EI24%) and Emulsification activity (EA).

Effect of Time Course on Bioemulsifier Production

Bioemulsifier productions by *Acinetobacter* AC5 was tested at different incubation period in MSM containing 1 % (v/v) of olive oil at pH 7 and incubated with continuous shaking (120 rpm) for 120 h. The biomass and E I 24% was measured for bioemulsifier production.

Effect of Inducer Oil and Hydrocarbon on Bioemulsifier Production

The effect on bioemulsifier production of different oils and hydrocarbons as a sole carbon source was assessed in 50 ml MSM supplemented with different oils such as: sun-flower oil, corn oil, olive oil, and sesame oil, as well as gasoline and burned diesel oil, at a concentration of 1% v/v, as a source of hydrocarbons.
To determine the best concentration of oil for bioemulsifier production, different concentrations of sesame oil (1% to 6%) were studied. The resulting cell-free supernatant was assayed for emulsification of each test oil and hydrocarbons according to EI 24% and emulsification activity.

Recovery of Bioemulsifier

Production of bioemulsifier was performed using the method described by Abouseoud *et al.*, (10); Khopade *et al*., (11). Briefly, *Acinetobacter* AC5 isolate was grown in 500 ml Erlenmeyer flasks, each containing 100 ml of MSM amended with 4% of seasame oil. The flasks were incubated at 30 ^{o}C in a shaker incubator for 72 h. To isolate the bioemulsifier, bacteria were precipitated by centrifugation at 10 000 rpm and 4ºC for 15 min. Bioemulsifier was precipitated from the supernatant by using different solvents systems: n-hexane, acetone, petroleum ether, diethyl either; and chloroform: methanol (2:1, v/v). The extract was filtered and the solvent evaporated and dried in an oven at 45 °C. The yellow to brown crude bioemulsifier was then weighed and used in the remaining studies. The solvent system showing the highest yield was used to recover the bioemulsifier from *Acinetobacter* isolates.

Chemical Analysis of Bioemulsifier

A portion of the partially purified bioemulsifier was used for chemical analysis. Carbohydrates were quantified according to the method proposed by Dubois *et al*., (12). Protein content was analyzed using serum albumin as standard according to the method described by Lowry *et al*., (13). Extraction and quantification of lipids was performed according to the method described by Reddy *et al*., (14).

Biomass Measurement

The dry weight technique was used to quantify microbial growth as bacterial dry weight. The biomass was first obtained after centrifugation at 10.000 g, 15 min, and then the precipitate cells were transferred to a weighed container, dried overnight at 105 ˚C, and reweighed.

Emulsification Activity (EA)

The emulsification activity of the culture sample was determined according to the method described by Patel and Desai, (15), with some modification. The emulsification activity was determined by taking 0.5 ml of cell free supernatant and adding it to 7.5 ml of 20 mM Tris-Mg buffer, supplemented with 0.1 ml of olive oil. This was then mixed in a high speed vortex mixer for 2 min. The tubes were left to stand for 1 h and absorbency was measured at 540 nm. Emulsification activity was defined as the measured optical absorbance.

Emulsification Index (EI24)

The E24% of the culture samples was determined by adding 2 ml of a hydrocarbon (toluene) to the same amount of culture free cells, mixing with a vortex mixer for 2 min, and leaving to stand for 24 h. The E24% is given as the height of the emulsified layer (mm) divided by the height of the hydrocarbon phase (mm), multiplied by 100 to give a percentage (16).

Antibacterial Activity

The antibacterial activity of the crude bioemulsifier was evaluated by the agar disc diffusion method according to Abouseoud *et al.*, (10) and Yalçin and Ergene, (3). The following bacteria were used to determine the antibacterial activity of the bioemulsifier: *E. coli, Salmonella sp., S. aureus and P. aeruginosa*. These bacteria were cultured in nutrient broth and incubated over - night at 37ºC. Serial dilutions (10^{-1} – 10^{-6}) were prepared, and 0.1 ml from the last dilution was spread on the nutrient agar plates. Sterile filter paper discs (0.6 cm) were soaked with bioemulsifier at three concentrations (10, 20 and 30) mg/ml in 0.1M (Tris-HCl) and distributed on the cultured medium. The plates were incubated at 37 ºC for 18 h where upon the inhibition in bacterial growth was observed around the discs.

Antifungal Activity

The effect of the bioemulsifier of *Acinetobactr* AC5 on the radial growth rate of *P. notatum* and *F. oxisporium* was studied on PDA. The sterilized growth medium was cooled down to 55 ^{o}C and amended with crude bioemulsifier to final concentrations of 0, 10, 20 and 30 mg/ ml, then the mixture mixed forcibly until it was homogenized, each plate containing 20 ml of growth medium. A plug (8 mm) of *P. notatum and F. oxisporium* mycelium, excised from the margin of PDA plates which had been cultured for 3 days, was placed in the center of the 9.0 cm diameter Petri plate containing PDA amended with partially purified bioemulsifier, and incubated at 28 ^{o}C. The radial growth rate was measured with a ruler after a further 3 days. Control plates contained growth medium without addition of bioemulsifier. The percentage of

radial growth inhibition was recorded according to the formula: Percentage of growth reduction = [(A-B)/A × 100], where: A= diameter of the control hyphal growth (mm), B = diameter of the treated hyphal growth (mm) (17). Controls were prepared with pure solvent (0.1M Tris-HCl) without addition of bioemulsifier.

Results and Discussion

Isolation and Identification of *Acinetobacter* Isolates

The ten *Acinetobacter* isolates from different sources had been previously identified to the genus level by morphological and biochemical tests (18), with the identification then being confirmed using API 20E system.
A microscopic examination of bacterial cells showed that they are Gram negative, diplococcobacilli, with cells in pairs, and no spore-forming. Biochemical tests showed that all isolates were negative for oxidase, indole, nitrate reduction, motility and IMVic and positive for catalase and citrate utilization.

Bioemulsifier Production by *Acinetobacter* Isolates

Out of ten *Acinetobacter* isolates, five showed evidence of lipase activity. All these lipase positive isolates were selected for further screening; with the *Acinetobacter* AC5 isolate being found to exhibit the strongest bioemulsifier activity, according to the results of E24% and Emulsification activity assessment (Table 1). This isolate was therefore selected for further identification and for bioemulsifier production in the remaining studies. The identification of isolate *Acinetobacter* AC5 was further confirmed by Vitek – 2 Compact Biomerieux (USA) and assigned as *Acinetobacter baumanii*. VITEK 2 is a new automatic system for identification and susceptibility testing of the most clinically and environmental important bacteria, and can also be used to identify all *S. maltophilia*, 91.8 % of *P. aeruginosa* isolates, and 76 % of *A. baumannii* isolates (19).

Table 1: Screening of isolates for bioemulsifier production in MSM containing 1% olive oil

No.	Isolates	Source of isolates	Lipase activity (mm)	EI24%	EA
1	AC 1	Contaminated soil	+	75	0.40
2	AC 5	Sediments	+	78	0.45
3	AC 6	polluted water	+	68	0.37
4	AC 8	Garden soil	+	50	0.30
5	AC 9	Garden soil	+	40	0.26

Time Course and Kinetics of Bioemulsifier Production by *Acinetobacter* AC5

The kinetics of bioemulsifier production and the growth pattern of AC5 are shown in (Figure 1). It can be observed that bioemulsifier production was associated with bacterial growth. Bioemulsifier production increased with cell biomass up to 72 h, and maximum bioemulsifier production was achieved at the end of the logarithmic phase (Figure 1). After 72 h however, bioemulsification activity decreased.

These characteristics suggest that the bioemulsifier is produced as a primary metabolite accompanying cellular biomass formation. The ratio of EI24% to cell biomass during production of bioemulsifier increased from 13.3 to 18.97 and then to 20.5 after 24, 48 and 72 h of incubation, respectively.

This result is in accordance with Amiriyan *et al*., (20), who reported the production of emulsan by *A. calcoaceticus* PTCC, IL-1 and PAY after 72 h of growth, with the total cell dry weights obtained being 3.6 and 4.8 g/l, respectively. The E24% results also increased with increasing cell growth, reaching an optimum of 75% at about 72 h before decreasing towards the end of the fermentation period. The production of an extracellular emulsifying agent by *A. calcoaceticus* sub sp. *anitratus* SM7 grown in MSM containing 0.3% n-hexadecane with an initial pH 7.0, at 30 ˚C has also been observed to be growth-associated up to 48 h (21).

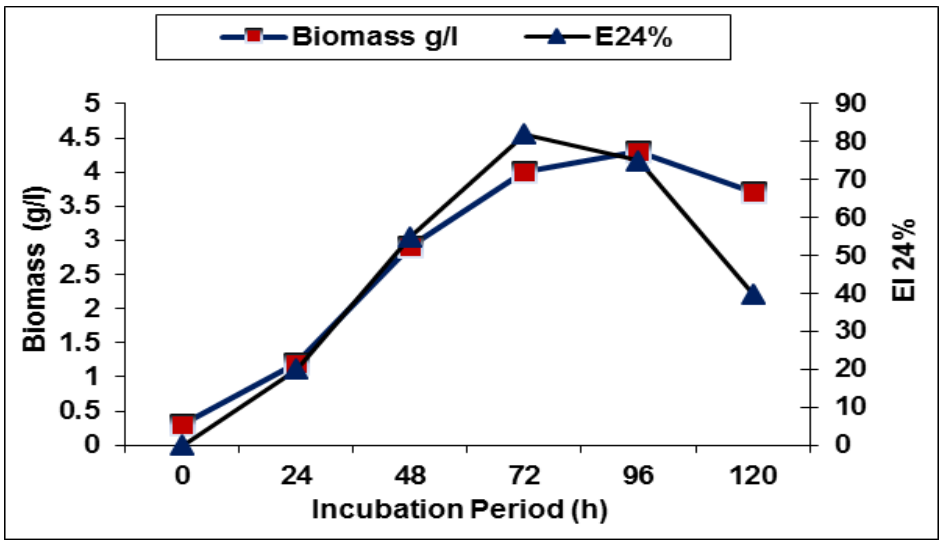

Figure 1: Incubation of *A. baumanii* AC 5 grown in mineral salt medium (pH7) with 1% olive oil at 30˚C

Effect of Inducer oil and Hydrocarbon on Bioemulsifier Production

The type, quality and quantity of bioemulsifier are influenced by the nature of the carbon substrate. Media containing edible oils and heavy oils were used for production of bioemulsifier from *A. baumanii* AC 5. In general the edible oils (sun flower oil, olive oil, sesame oil, corn oil) were found to be more suitable for bioemulsifier production. Out of the different vegetable and edible oils and hydrocarbons, the most bioemulsifier was produced with sesame oil as the carbon source, yielding an EI24% of 83% and an EA of 0.36 (Figure 2). Only Slight emulsifying activity was observed when gasoline and diesel oil were used.

Determine of the optimum bioemulsifier production from *A. baumanii* AC 5, media containing different concentrations of sesame oil were used. The results showed that the ability of the isolate to tolerate up to 6% of oil, although the maximum bioemulsifier production (EI24% = 89% and EA 0.35) was achieved with a sesame oil concentration of 4% **(**Figure 3). The growth of microorganisms and bioemulsifier production was reduced at oil concentration over 4%.

According to bioemulsifier production, most reports have suggested, the necessity of supplementation with oils and hydrocarbons to induce production. Hydrophobic substrates like corn oil, lard (which are rich in unsaturated and saturated fat) and long chain alcohols induce microbial growth and metabolite production owing to their typical fatty acid composition by maximizing biosurfactant production (22).

Bacteria consume edible oil more easily than heavy oil because the heavy oils are more complex and may be toxic and difficulty to utilize as carbon source. The lower toxicity of vegetable oils when used as a carbon makes them good inducers for bioemulsifier production (23). This observation is in accordance with the bioemulsifier production from *A. lowffii* TA 38 (24). Of the six oils tested, castor oil was found to be emulsified the most, while soybean oil was least emulsified.

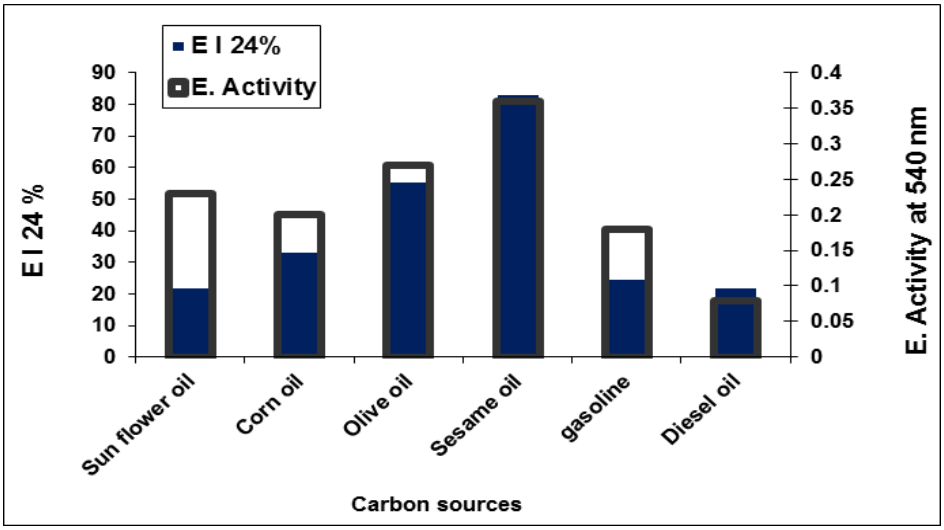

Figure 2: Effect of different oil sources on bioemulsifier production of *A. baumanii* at 30°C, pH7 in a shaker incubator at 120 rpm, 30 °C and pH7 for 72 h

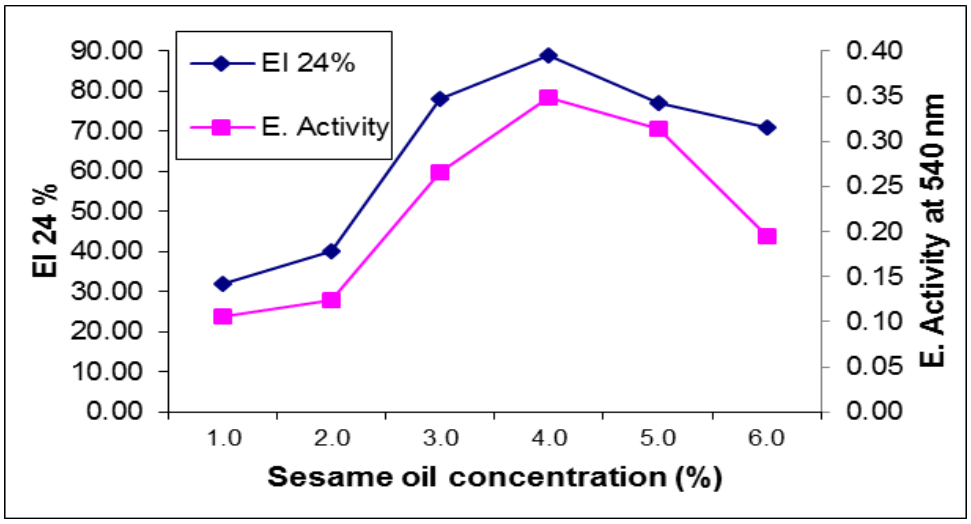

Figure 3: Effect of different concentration of sesame oil on bioemulsifier production by *A. baumanii* AC5 in a shaker incubator at 120 rpm, 30 °C and pH 7 for 72 h

These results are also in agreement with Amiriyan *et al*., (20), who observed, the highest emulsion values (water-in-oil) of about 78% by *A. calcoaceticus* using crude oil. Also the results of the current study observed minimum emulsification activity when gasoline and diesel oil were used as the sole source of carbon. *Acinetobacter* Ud-4 possesses a strong capacity to degrade edible oils (canola oil, olive oil, sesame oil, soybean oil, and lard), the strain grew well and heavily degraded edible oils over seven

days at 25 °C (25). The isolate *A. baumanii* AC 5 was observed to tolerate up to 6% sesame oil, but the optimum bioemulsifier production (EI24% 89% and EA 0.35), was obtained in the presence of 4% of oil (Figure 3). This observation was similar to that obtained by Patil and Chopade (26), who mentioned that the ability of strain *A. junii* SC14 to tolerate up to 40% almond oil, but the optimum bioemulsifier production took place in the presence of 18% almond oil (427.8 EU/ml).

Recovery of Bioemulsifier

Five different organic solvents were used to extract the bioemulsifier produced by *A. baumanii* AC5. Of these five precipitation methods, chloroform: methanol (2:1) was the most efficient in recovering bioemulsifier from the cell-free supernatant of AC 5 culture grown in the presence of 4% sesame oil (Figure 4). Recovery yields of 5.05 g/l, 3.2g/l, 2.03g/l, 0.8 g/l and 0.5 g/l were obtained when chloroform: methanol, diethyl ether, n-hexane, petroleum ether and acetone were used respectively. For this reason the solvent chloroform: methanol, was followed used in the subsequent the extraction and partial purification of Maneerat and Dikit (27) they showed in their study that the mixture of chloroform: methanol (1:1 v/v) was the most efficient in extracting the cell-associated bioemulsifier from a cell suspension of *Myroides* sp. SM1. Phetrong *et al*., (21) recovered the bioemulsifier from the culture supernatant of *A. calcoaceticus* sub sp. *anitratus*. They observed a maximum yield 2.94 g/l with ethanol precipitation, and lower yield obtained with acetone.

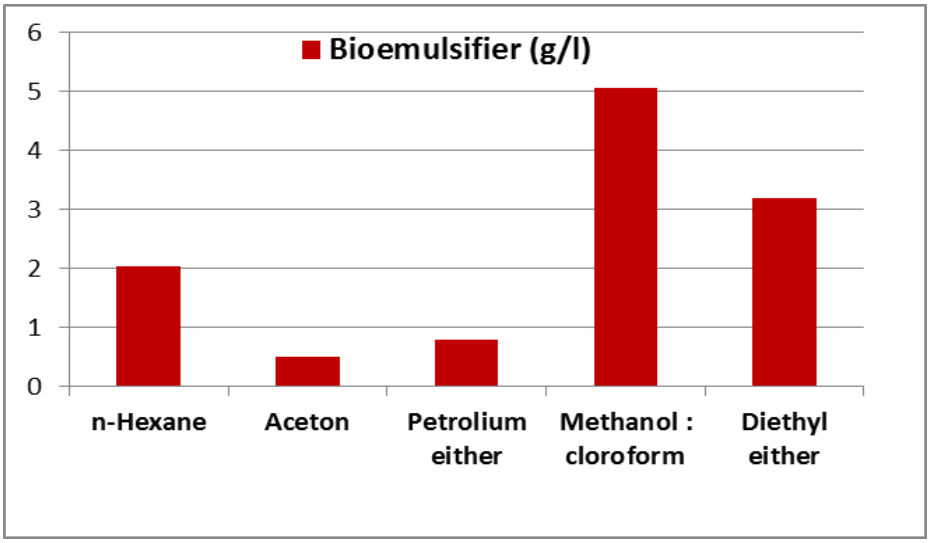

Figure 4: Extraction of bioemulsifier produced by *A. baumanii* AC5 using different solvent system

Chemical Composition of Bioemulsifier

The bioemulsifier produced by AC5 was yellow to brown in color, and with an oily nature after drying. The partially purified bioemulsifier gave a yield of 5.05 g/l under the given conditions. Chemical analysis of this bioemulsifier revealed lipid (63%) was the major constitute, followed by carbohydrate (35%). A minor fraction of protein (2%) was also detected in the bioemulsifier. This lipoglycan nature of the bioemulsifier produced by *A. baumanii* AC5 is evident from its chemical composition.

Bioemulsifiers may be lipopolysaccharides, lipoproteins or combinations of these. The hydrophobic moiety necessary for emulsification can be a protein, as in BD4 emulsan from *A. calcoaceticus* BD4, a carbohydrate, as in liposan from *Candida lipolytica* or a lipid, as in the emulsan from *A. calcoaceticus* RAG-1 (28). The emulsan produced by *A. calcoaceticus* RAG 1 has been shown to be an extracellular no covalent complex of a lipopolysaccharide and a protein (29). A proteoglycan bioemulsifier with maximum yield of 3.9 g/l produced by *A. junii* SC14 has also been reported (24), while, Navon-Venezia *et al.*, (30) produced 4.6 g/l of alasan by *A. radioresistens*.

Antibacterial Activity of Crude Bioemulsifier

The antimicrobial activity of bioemulsifier was examined against *E. coli, Salmonella sp., S. aureus and P. aeruginosa.* It was observed that the bioemulsifier reduced the growth of these bacteria, with the greatest effect evident on the growth of *S. aureus,* followed by *P. aeruginosa, and E. coli* and *Salmonella sp.*, respectively (Table 2 and Figure 5).

Comparing these results with those from other studies, the bioemulsifier produced by *Streptomyces* sp. showed a wide activity against pathogenic strains, with the partially purified bioemulsifier showing the highest activity against *S. aureus, P aeruginosa, B. subtilis and E. coli* (11). The rhamnolipids from *P. aeruginosa* ATCC 9027 are also active compounds with antibacterial activity. Mono-rhamnolipid at a concentration of 4 g/L (1.3 g/L rhamnose) led to inhibition of the growth of *Mycobacterium aurum* (31).

Table 2: Antibacterial activity of AC5 bioemulsifier against pathogenic bacteria in nutrient agar after 18 h of incubation at 37 °C

Concentration of Crude bioemulsifier (mg /ml)	Zone of Inhibition (mm)			
	E. coli	*Salmonella sp.*	*S. aureus*	*P. aeruginosa*
10	26.5	19.3	21.5	15.35
20	33.4	28.8	26.75	25.5
30	35.2	29.9	43.7	38.96

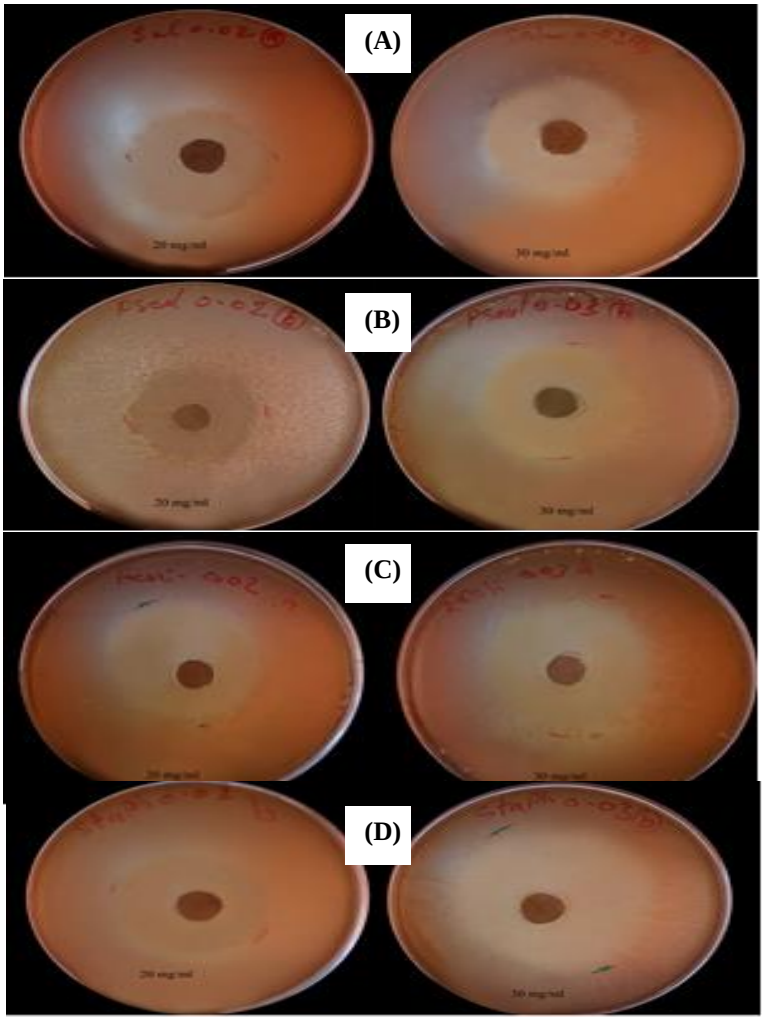

Figure 5: Antibacterial activity of bioemulsifier produced by *A. baumanii* AC5 at a concentration of 20 and 30 mg/ml, in nutrient agar: A- *E. coli*, B- *P. aeruginosa*, C- *Salmonella sp.*, and D- *S. aureus*

Antifungal Activity of Crude Bioemulsifier

The antifungal activity of partial purified crude bioemulsifier and their effects were studied on potato dextrose agar (PDA) by determining the radial growth rate and percentage of growth reduction. It was found that the partially purified bioemulsifier inhibited the radial growth rate of *P. notatum* and *F. oxisporium*. The antifungal activity increased with increasing concentrations of bioemulsifier. The reduction in the radial growth rate ranged from 22.78 to 55.39% and from 46.52 to 64.34 % for *P. notatum* and *F. oxisporium* respectively at a bioemulsifier concentration of 10 to 30 mg / ml (Table 3 and Figure 6).

One of the main modes of bioemulsifier action, including rhamnolipids and cyclic lipopeptides (CLP) involves the formation of ion channels in the plasma membrane of the target organisms leading to cytolysis (3).

Table 3: The radial growth rate and reduction percentage of radial growth for *P. notatum* and *F. oxisporium* cultivated on PDA amended with different concentrations of AC5 crude bioemulsifier after 72 h of cultivation

Concentration of Crude bioemulsifier (mg /ml)	Radial growth rate (mm)		Reduction (%)	
	P. notatum	***F. oxisporium***	***P. notatum***	***F. oxisporium***
0 (control)	66.7	46.0	0	0
10	51.5	24.6	22.78	46.52
20	45.7	21.1	31.48	54.13
30	29.75	16.4	55.39	64.34

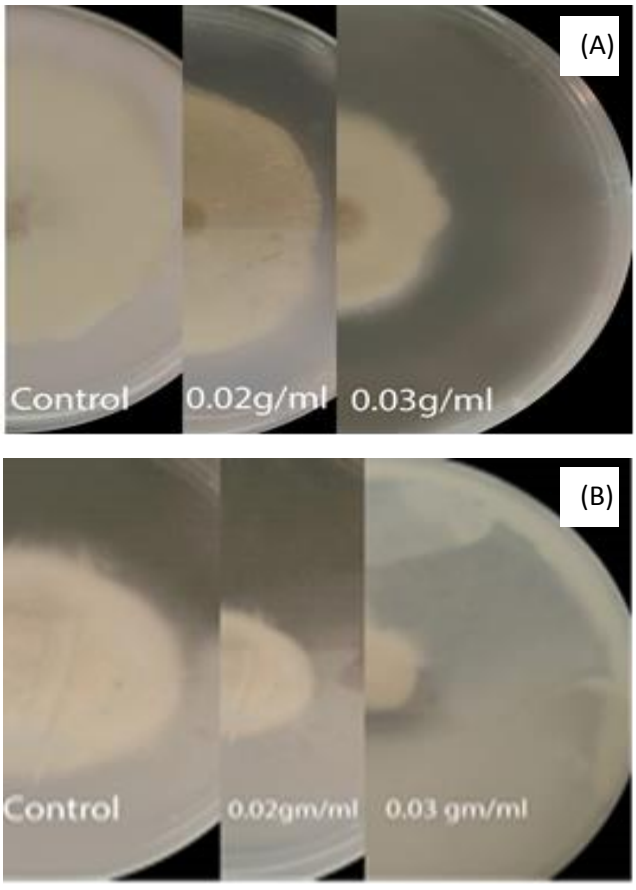

Figure 6: Antifungal activity of bioemulsifier produced by *A. baumanii* AC5 at a concentration of 20 and 30 mg/ml against: (A) *P. notatum*, (B) *F. oxisporium*

Since fungi possess a cell wall composed largely of carbohydrate layers and long chains of polysaccharides, as well as glycoprotein and lipids (32), bioemulsifier can act on the lipids in the cell wall, altering the permeability of the membrane and perhaps producing pores through which ions and small molecules are lost. Cao *et al*., (33), studied the antifungal activities of the crude lipopeptide biosurfactant from *Bacillus natto* ASA at different concentrations (0.8-3.2 g/L) using the disc diffusion method. The biosurfactant exhibited increasing antifungal activities, in that the antifungal activity increased with increasing concentrations of biosurfactant until a maximum zone of inhibition was obtained (48.8 mm) with *Botrytis cinerea* at a biosurfactant concentration of 3.2 g/l. Nielsen *et al*., (34), evaluated the role of CLP compounds produced by fluorescent *Pseudomonas* sp. in the biological control of pathogenic microfungi (*Rhizoctonia solani*) on PDA media. The results demonstrated that the highest level of antagonism was obtained against microfungi, with a zone of inhibition of 26 mm in comparison to a zone of control of 60 mm. Mazzola *et al*., (35), mentioned the effect of cyclic lipopeptide surfactant produced by *P. fluorescence* SS101 for suppression of complex *Pythium* spp. on nutrient broth yeast, amended with partially purified (70%) cyclic lipopeptide (CLP). The maximum reduction in radial growth was obtained at 72 h incubation, and ranged from 46-96% among *Pythium* spp. isolates at a CLP concentration of (500 μg /ml).

Conclusion

Out of ten *Acinetobacter* isolates screened for bioemulsifier production, five isolates were showed ability to emulsify olives oil. The bioemulsifier production was associated with bacterial growth, and that the presence of inducer edible oils in the medium also enhanced bioemulsifier production. Chemical analysis of the bioemulsifier revealed that it is a lipoglycan in nature mainly contained lipid and carbohydrate and a minor fraction of protein. The crude bioemulsifier; showed strong antimicrobial and antifungal activity.

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