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Production of Extracellular Glycoprotein Biosurfactant from *Rhodotorula glutinis* and Its Use in Elimination of Solar Pollution

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THE SEARCH for cheaper biossurfactant production alternatives has attracted the recent studies. Yeast isolates revealing biosurfactant activities were obtained from autochthonous microflora of asphalt heavily polluted with solar slurries collected from five different bus garage stations in Cairo. By means of standard yeast identification procedures the isolates were identified. Seven yeasts were isolated from fifty localities, they were *Candida guilliermondii, Candida kefyr, Candida tropicalis, Pichia* sp, *Rhodotorula glutinis, Saccharomyces* sp and *Torulopsis* sp. The preliminary investigation of the extracellular biosurfactants production was detected by using three parameters. The culture of *Rhodotorula glutinis* gave the highest biosurfactant activity. Optimal conditions for maximum biosurfactant activity were investigated under different temperatures; pH values; different sources of phosphorus, nitrogen and different concentrations of yeast extract. Anaytical methods for the purified biosurfactant showed that the biosurfactant is glycoprotein. Electrophoretic profile showed ten bands in the protein portion of the purified biosurfactant . Three amino acids were detected in the protein moiety.The possibility of the biosurfactant for commercial application was studied by Sandpack test .

Keywords: Biosurfactant , Yeasts- Solar , Glycoprotein.

Pollution of sewage with oil spills from car stations and others from oil used factories as fuels is consider a big problem in recent years. Microorganisms synthesize a wide variety of high- and low-molecular-mass bioemulsifiers (Rosenberg and Ron 1997; Richter *et al.* 1998 & Rahman *et al.* 2002). Microbial surfactants are a diverse group of surface-active structures which have attracted a considerable interest in recent years, due to their potential commercial applications in detergent industry (Rosenberg and Ron 1998, 1999), formulations of herbicides and pesticides (Rubinovitz *et al.* 1982 & Patel and Gopinathan 1986), petroleum, pharmaceutical, food processing and capacity to be manufactured from renewable and cheaper substrates (Maier and Soberon-Chavez 2000; La Duc *et al.* 2004 and Cameotra and Makkar 2004).

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Biosurfactant has unique amphipathic properties derived from their complex structures, which include a hydrophilic moiety and a fuel hydrophobic portion. The lipopeptides and glycoprotein are interesting classes of microbial surfactants (Cameotra and Makkar 1998 & Walzer *et al.* 2006) because of their manifold attractive properties. Biosurfactants produced by hydrocarbon-degrading microorganisms can emulsify hydrocarbon-water mixtures, which enables them to grow on the oil droplets. These emulsification properties have also been demonstrated to enhance hydrocarbon degradation in the environment, making them potential tools for oil spill pollution-control (Banat 1995). Biosurfactants are used for soil washing or flushing due to their ability to mobilize contaminants. (Lin 1996). The hydrophilic portion of biosurfactant can be a carbohydrate, amino acid, cyclic peptide, phosphate, carboxylic acid or alcohol. They can be potentially as effective with some distinct advantages over the highly used synthetic surfactants including high specificity, biodegradability and biocompatibility (Kanga *et al.* 1997). Fermentative production, composition and yields of biosurfactants by microorganisms depends primarily on microbial strain, sources of carbon & nitrogen, pH, temperature, concentration of oxygen and metal ions in the growth medium(Mulligan and Gibbs 1993).

The choice of biosurfactant is primarily based on its advantages; low cost, low toxicity and biodegradable ability (Mulligan and Gibbs 1993). The emulsifying agents are produced only in the presence of the water-immiscible substrates and appear to facilitate their metabolism (Pan *et al.* 1999 & Maker and Cameatra 2002). Fortunately, some indigenous yeasts from chronically contaminated sediment may produce biosurfactant (Jonathan *et al.* 2003). The bioemulsifiers were extracellular or bound to the cell surface, and often contained carbohydrate and peptide material (Cameron *et al.* 1988). The present work is a trial to isolate local yeast from solar contaminated soil and the investigation of isolates abilities to produce extracellular biosurfactant on synthetic medium containing solar as a carbon source. Purification and characterization of the biosurfactant will be also elucidated high yield of such environmental friend.

Material and Methods

Chemicals

Crude solar was obtained from Enby Company for chemical industries, Nasr City, Cairo. All other fine chemicals were purchased from Sigma Company for chemicals.

Isolation of biosurfactant producing yeasts

The yeast strains were isolated from microflora present in oil slurry-contaminated soil in asphalt from five different bus garage stations in Cairo according to the procedure of Kaszycki *et al.*, (2006). 10g sample of ten different loci of solar polluted soil was suspended in 90 ml of distilled sterile water and incubated for 3 hr at room temperature in 500 ml flasks in a laboratory rotary shaker (200 r.p.m). Then the soil, and other solid particles were filtered out

under aseptic conditions; the remaining suspension was enriched with a minimum amount of bacto-peptone and yeast extract (0.5 g/l for each) and left for 2 hr. To isolate yeast, appropriate dilutions of the original suspension were spread onto Petri dishes with solid Sabouraud medium. After growth at 30 °C ± 2°C for 2 days single colonies were picked based on their morphology and microscopic observations and re-plated onto solid media containing a mixture of antibiotics (40 mg/l streptomycin and 28 mg/l penicillin). The isolated yeast strains were stored on solid agar slants at 4°C for further studies.

Screening of biosurfactant producing yeast

Thirty different isolates of slimy, yellow and red colonies were tested for the ability to produce biosurfactants. The preliminary investigation of biosurfactants production was detected by using three parameters ; the formation of a clear zone on blood agar plates for 2 days (Morikawa *et al.* 1993), the formation of a clear zone on solar (1 %) - $CaCl_2$ agar plate examined daily up to 10 days (Wohlfarth and Winkler 1988) and by the quantitative drop-collapse method (Bodour and Miller-Maier 1998). The culture of *Rhodotorula glutinis* which gave the highest biosurfactant activity was maintained on agar slants of the same media at 4°C for the emulsifier production studies .

Emulsification activity

Emulsification activity was performed according to emulsification index (E_{24}%) and confirmed by the drope collapse method. Emulsification index (E_{24}%) measured according to the method described by Paraszkiewiczk *et al.*, (2002). Solar was added to the culture supernatant after centrifugation of cells at 15,000 × *g* for 10 min at 4°C in a ratio of 3:2 and then vortexed for 1 min. After 24 hours, the emulsification index (E_{24}) was estimated by the height of the emulsification layer, divided by the total height of the system multiplied by 100. All assays were performed in four replicates for each treatement . A control experiment with no yeast growth was also estimated.

Drop-collapse method

Drop-collapse method was performed according to the procedure described by Bodour and Miller-Maier (1998). Brifely, The assay is performed in a 96-microwell plate, where each well is thinly coated with 2 µl of oil then 5 ml sample droplet is added to the center of a well and observed after 1 min with dissecting microscope to measure the diameter of the droplet.

Identification of the yeast

The isolated yeast was identified by means of standard microbiological methods (API 20 C AUX, YEAST) based on: (a) morphological features and (b) biochemical characteristics like carbohydrate fermentation as well as, assimilation of carbohydrate and nitrogen compounds.

Growth determination

ATP was used as specific indicator for monitoring the biomass of the yeast according to the method described by Forsberg and Lam (1977).

Growth conditions for biosurfactant production

The isolated *Rhodotorula glutinis* was grown aerobically on minimal salt medium of Albuquerque *et al.*,(2006) with the following composition (g/100ml): 0.8 urea, 0.22 ammonium sulfate, 0.8 potassium dihydrogen phosphate, 100 ml of distilled water. Solar was added at different concentration (g/100 ml) (w/v) (0.05,0.1,0.2,.0.4,.0.6,0.8,1.0,1.2). The pH of the medium was adjusted at pH 5.0 then autoclaved at 120°C for 15 min. Twenty mls of the standard inoculum ($5x10^7$ cell / ml) were introduced into 2-liter Erlenmeyer flasks containing 800 ml of medium with shaking at 200 rpm in a shaking incubator at 30°C and the productivity was estimated within 10 days .

Effect of different culture conditions on biosurfactant activity

The productivity of biosurfactant was studied at different temperatures; different pH values; different sources of phosphorus and nitrogen sources, different concentrations of yeast extract and different concentrations of phosphorus source. Four riplicate flasks were used for each treatement .

Surfactant isolation and purification

Emulsifier production was carried out at optimum conditions. The highest productivity was obtained by introducing the growth in 2-liter Erlenmeyer flasks containing 800 ml of medium in shaking incubator at 30°C. Yeast growth was initiated by introducing twenty mls of the standard inoculum ($5x10^7$ cell / ml) obtained from a starting culture, that was grown in 150 ml of modified minimal medium at optimum conditions, for producing biosurfactant in a 1000 ml flask at 30°C ± 2 °C for eight days. Growth was detected by the determination of ATP as described in methods. The dissolved oxygen level was kept above 20 % of air saturation by increasing rotation speed up to 750 rpm. Foam was controlled by adding antifoam 289b (Sigma, ST, and Louis. MO). After the end of incubation period the culture was centrifuged at 15.000 × g for 10 min at 4°C to obtain cell free supernatant containing extracellular biosurfactant. The crude cell-free supernatant was divided into two parts. The resulting first filtrate was filtered at 25°C through a 0.45-,um membrane (Millipore Corp.). The cell-free filtrate was transferred to a 2-feet (61-cm) length of dialysis tube (diameter, 4 cm) and concentrated to 50 ml by prevaporation at 4°C (Cirigliano and Carman. 1984). The concentrated filtrate (50 ml) was extracted with 500 ml of chlorofom methanol (2:1, v/v) in a 1-liter separating funnel at 25°C as previously described (Bligh and Dyer 1975). The aqueous phase was reextracted two times with 500 ml of chloroform-methanol (2:1); .The aqueous phase which may has lipids after the three extraction was tested for emulsification activity .The second part of the filtrate was precipitated by ammonium sulfate (60% saturation), followed by exhaustive dialysis against 1,000 volumes of distilled water for 24 hr (fresh water was replaced twice). The polymer produced was stored as a lyophilized powder at room temperature and tested for emulsification activity, carbohydrate, fats and protein after dissolving (4: 2 ,w : v) in 20 mM Tris hydrochloride buffer (pH 5) .

Properties of the emulsions

Stabilization of emulsions by purified emulsifier was evaluated over a range of chemical and physical conditions. Purified emulsifier was dissolved in distilled water, and the pH was adjusted to a range between 3 and 10 with HCl or KOH and the effect of different temperatures was also detected. After oil was added, tubes were vortexed and the emulsions were measured. For the evaluation of stability, emulsions containing 0.1% (w/v) emulsifier in distilled water and hydrocarbon were incubated at 30°C and its ability to emulsify hydrocarbons and organic solvents was tested.

Analytical methods

The purified biosurfactant was analyzed for its content of protein, carbohydrate and lipids by the methods of Bradford (1976), Dubois *et al.*, (1956) and Makula *et al.*, (1975) respectively.

Preparation of deproteinized biosurfactant

Apo-biosurfactant (deproteinized biosurfactant) was obtained by the hot-phenol method (Westphal and Jann. 1965).

Sandpack test

The sandpack test was performed according to the procedure of Banat *et al.*, (1991). Glass columns (50 x 2.5cm) were packed with acid washed sand saturated with 10 ml of solar. Hundered mls of biosurfactant solution (0.5% , 1%, 2%, 3%) w/v biosurfactant : water) was added to the sulphuric acid washed sand. Recovery of the oil was estimated by measuring the turbidity of recoverd solution and compared with control .

SDS-PAGE

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed as described previously by Laemmli, (1970) in a Hoffer apparatus. Protein bands were stained with Coomassie brilliant blue G-250. The banding profile was photographed. The number of bands was scored and the recording data was computerized and analyzed by Gel proanalyzer version 2 soft ware in the central lab., Department of Biological Sciences and Geology, Faculty of Education at Ain Shams University.

Amino acid analysis

The biosurfactant was hydrolyzed with distilled 6 N HCl (containing 0.2% phenol) at 110°C for 24 hr. Thin-layer chromatography (TLC) was carried out on 20- by 20-cm precoated cellulose plates (Sigma). Hydrolyzed samples of the biopolymer were developed with ethyle acetate: pyridine: water: acetic acid (5:5:3:1 by volume). After air drying, the chromatograms were stained for amino acids (Gal 1968) by spraying the plates with 0.2% ninhydrin in acetone followed by heating at 105°C for 5 min.

Statistical analysis

All the data were subjected to T- test using the Statistical analysis system SPSS version 14, Correlation coefficient was also conducted to determine the

relationship between the growth and biosurfactant synthesis. Only significant values at $p < 0.05$ were considered for representation and discussion .

Results and Discussion

Thirty different yeasts were isolated from soil polluted with solar. The preliminary investigation Table 1 showed that only 7 isolates produced relatively active biosurfactant detected by the formation of clear zone on both blood agar and on solar (2%) Ca Cl2 agar plate. The isolates which have biosurfactant activity were checked by the oil displacement method, this method is enabled assay to detect the biosurfactant productivity to at least 10 µg / ml. (Morikawa *et al.* 1993). These producers' isolates were identificated by the method described by API system for yeast identification. The results of biochemoical and morphological characteristics showed that they were *Candida tropicalis Candida guilliermondii, Candida kefyr*, *Pichia* sp, *Rhodotorula glutinis, Torulopsis* sp and *Saccharomyces* sp. In this regard, it was reported that the growth of microorganisms on hydrocarbon containing medium is limited by a decrease of interfacial tension and increase in the degree of medium emulsification as critical factor (Palittapongarnpim *et al.* 1998). In environmental biotechnology, genera such as *Pichia*, *Hansenula*, *Candida*, *Yarrowia* and *Trichosporon* are of special importance in oil biodegradation and biosurfactant production (Mukherjee *et al.* 2006 and Międzobrodzki *et al.* 2006). In the same regard, two different strains of yeasts were isolated with high surface activity from 1000 samples from soil, sludge , waste water producing biosurfactant (Pan *et al.* 1999). In the same respect, among 200 yeast strains isolated from plants in Thailand, 50 strains produced relatively high lipase and 30 of them were biosurfactant producers. (Thanomsub *et al.* 2004).

TABLE 1. Percentage of positive biosurfactant activity of yeast isolates for different periods on mineral oil basal medium.

Yeasts	Percentage of positive isolates in the collected samples at different periods		
	1-3 days	4-6 days	7-10 days
Candida tropicalis	8	11	11
Candida guilliermondii	2	3	3
Candida kefyr	5	6	6
Pichia sp	4	5	5
Rhodotorula glutinis	22	25	25
Torulopsis sp	7	8	8
Saccharomyces sp	1	0	0

Biosurfactant production by Rhodotorula glutinis as influenced by different levels of solar in the growth medium

For detection of extracellular biosurfactants in growth culture broth, *Rhodotorula glutinis* was grown in the minimal medium fortified with different concentrations of solar (0.1-1.2 g/100 ml). The E_{24} peaks (58%) of isolated strain of *Rhodotorula glutinis* were found maximum after incubation period for 8 days at 0.8 solar (g/100 ml) (w/v) (Fig. 1). In this respect, Paraszkiewicz *et al.*, (2002) found that the maximum E_{24} peaks (93.0 % and 81.0 %) at 24 and 56 hours for the biosurfactant producer fungus *Curvularia lunata.* On contrary, gram-positive bacteria biosurfactant producer grown on oil diesel as a carbon source showed only 12% of emulsification (Cassidy and Hudack 2001). This methodology proved to be cheap and effective for the screening, maintenance and quantification of biosurfactant-producing microorganisms and was recommended for tropical countries with low founding resources (Krepsky *et al.* 2007) .

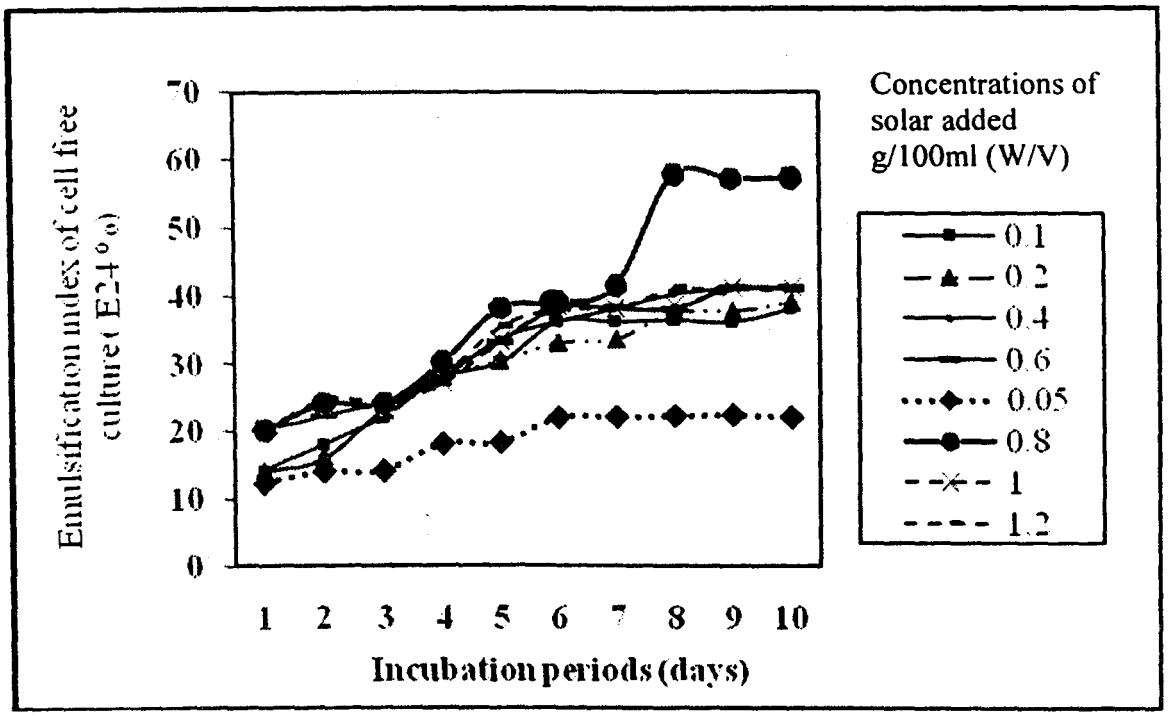

Fig. 1. Emulsification index (E24 %) of cell free culture of *Rhodotorula glutinis* at different concentrations of solar for different incubation periods.

Factors control the production of extracellular biosurfactant

The previous studies had led to the conclusion that the biosurfactant is produced in a response to the insoluble substrate to emulsify or disperse this phase into the water and make it available to the organisms (Nakahara *et al.* 1977). So, suplementation of the medium with other simple carbon source may help the organism to assimilate the hydrocarbon. Different carbon sources were added to the minimal medium containing 0.8 g % solar (w/ v). Results in Figs. 2 & 3 showed that 1.5% glucose increased the growth and the biosurfactant productivity of *Rhodotorula glutinis.* Similarly, *Bacillus subtilis* produced high

yields of a very active surfactant when the growth medium contained a carbohydrate as carbon source (Cooper and Paddock 1983). In the same trend, Lee and kim (1999) emphasized that glucose is one of the most convenient carbon source for biosurfactant production. Chen *et al.*, (2007) mentioned that 4% glucose could attain better rhamnolipid yield. Recently, Saruboo *et al.*, (2007) stated that the addition of glucose to the minimal medium containing canola oil increased the biosurfactant abilities of *Candida lipolytica.*

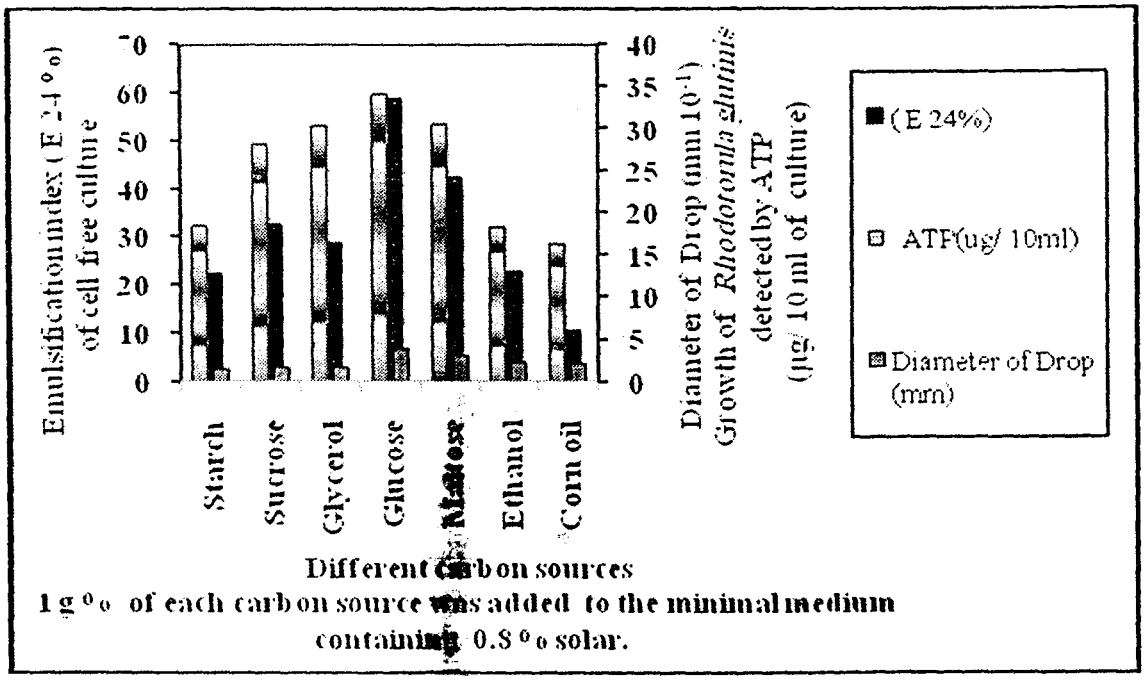

Fig. 2. Effect of different carbon sources on the extracellular production of biosurfactant and growth of *Rhodotorula glutinis* .

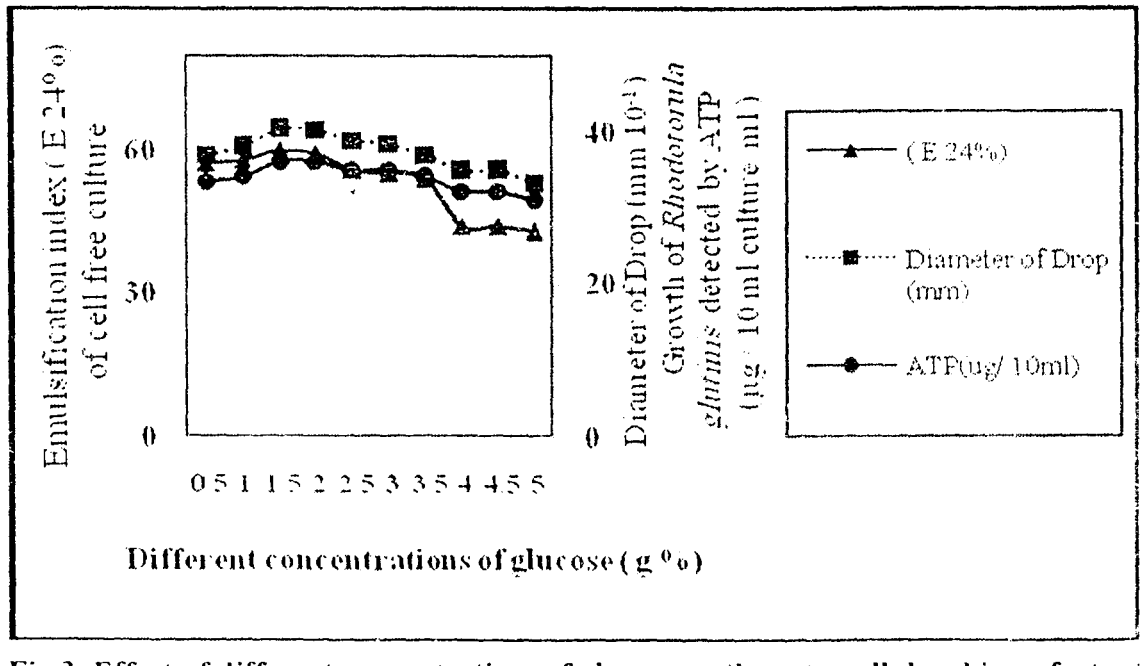

Fig.3. Effect of different concentrations of glucose on the extracellular biosurfactant production and growth of *Rhodotorula glutinis* .

To determine the optimal nitrogen source for biosurfactant activity, urea was replaced in the minimal medium with either of ammonium sulfate, ammonium nitrate, ammonium chloride, glycine, glutamic acid or arginine on the basis of the same equivalent weight. The results in Figs. 4, 5 showed that urea was the most preferable nitrogen source at 0.8 g % for both growth and extracellular biosurfactant production. In this respect , Desai and Banat (1997) stated that the addition of water-immiscible substrates and nitrogen in the media result in an overproduction of some biosurfactants. In the same respect , Albuquerque *et al* ., (2006) stated that 8 g/l urea was the optimum concentration for maximum biosurfactant production by *Candida lipolytica.*

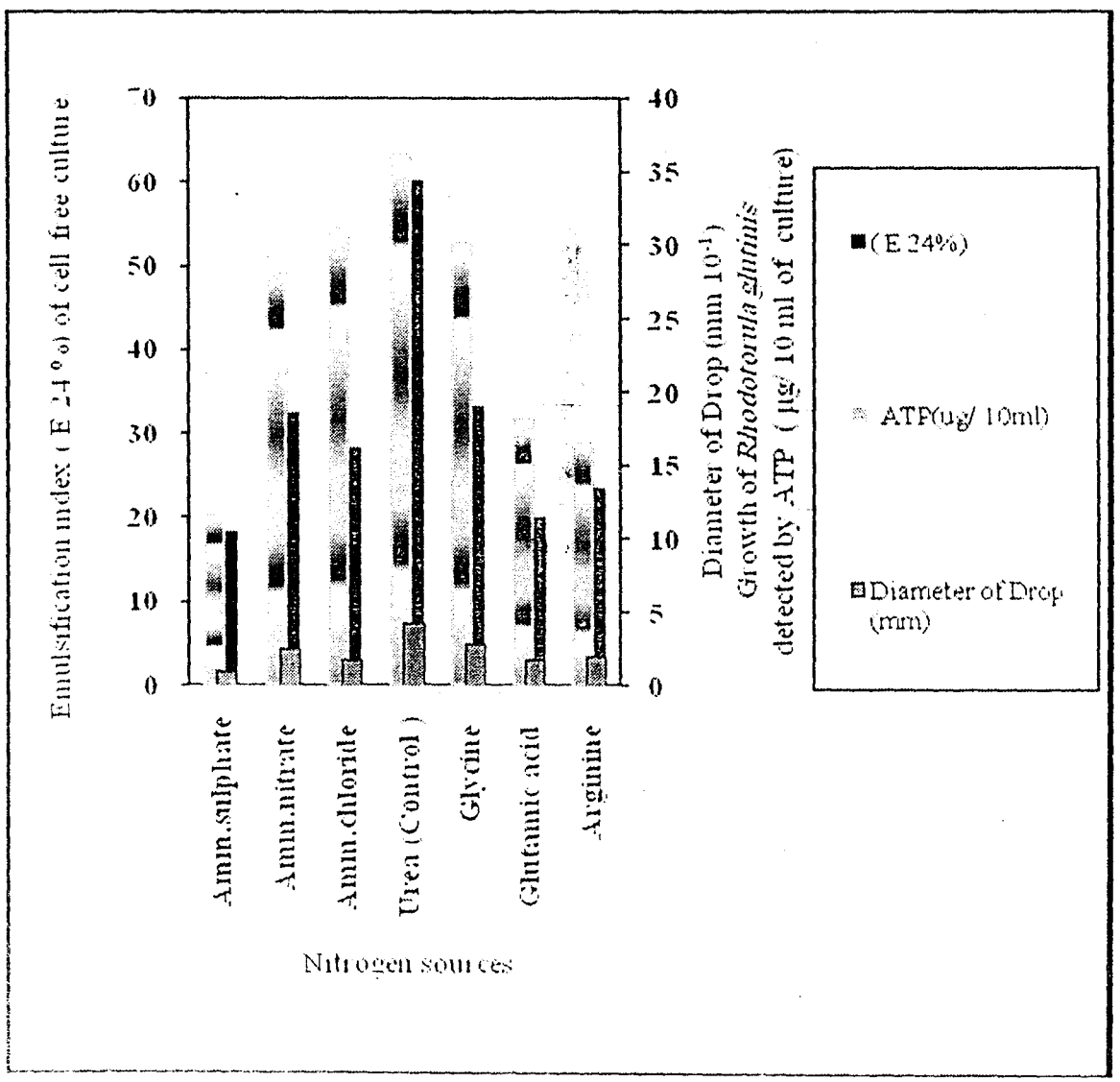

Fig. 4. Effect of different nitrogen sources on the extracellular production of biosurfactant and growth of *Rhodotorula glutinis.*

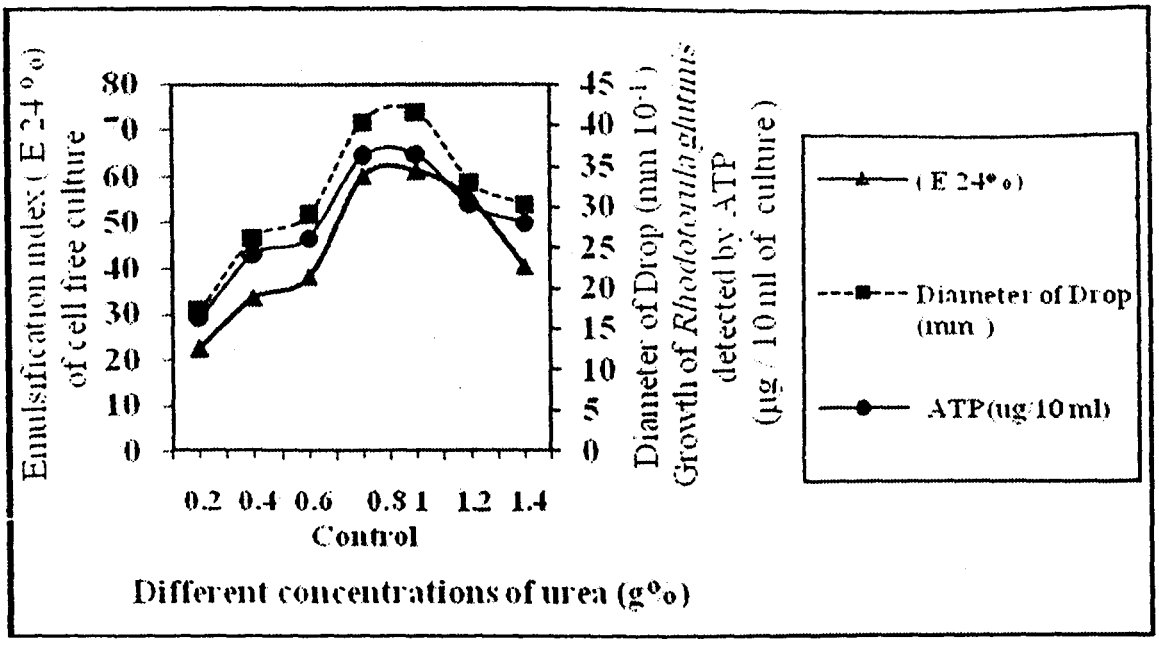

Fig. 5. Effect of different concentrations of urea on the extracellular biosurfactant production and growth of *Rhodotorula glutinis*.

Results shown in Figs. 6 & 7 revealed that of potassium dihydrogen phosphate at 8 g/L was the optimum concentration for enhancement of biosurfactant production by *Rhodotorula glutinis*. In this regard, Cha *et al.*, (2004) stated that 0.065% of potassium dihydrogen phosphate was the optiumum for maximum production of glutathione by *Saccharomyces cerevisiae* FF-8.

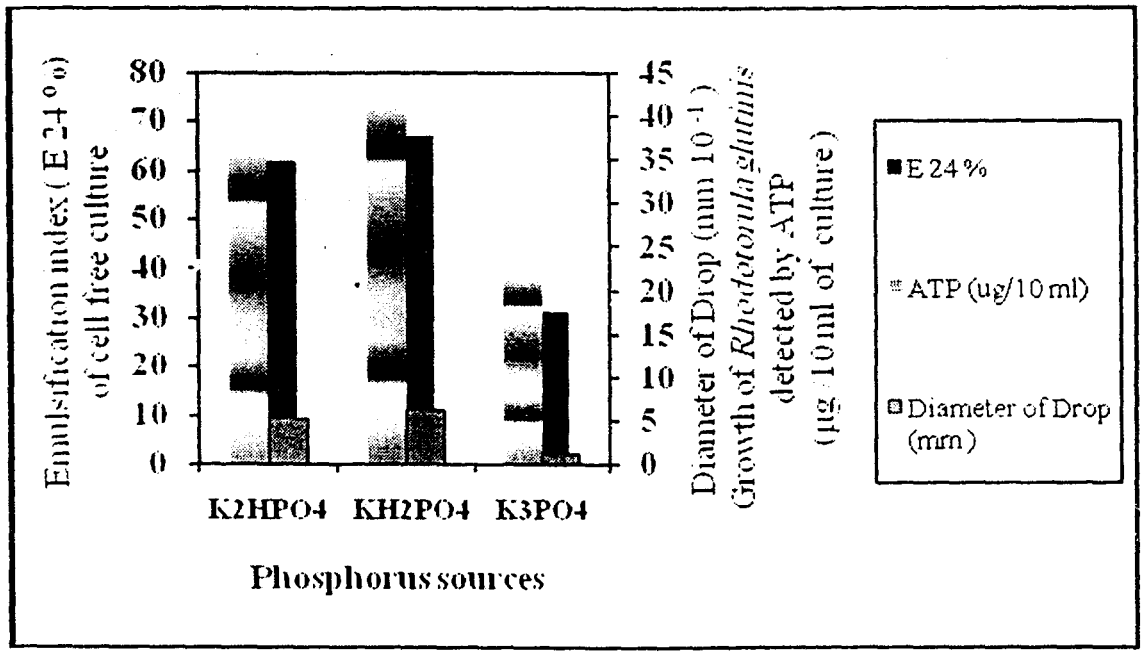

Fig. 6. Effect of different phosphorus sources on the extracellular production of biosurfactant and growth of *Rhodotorula glutinis*.

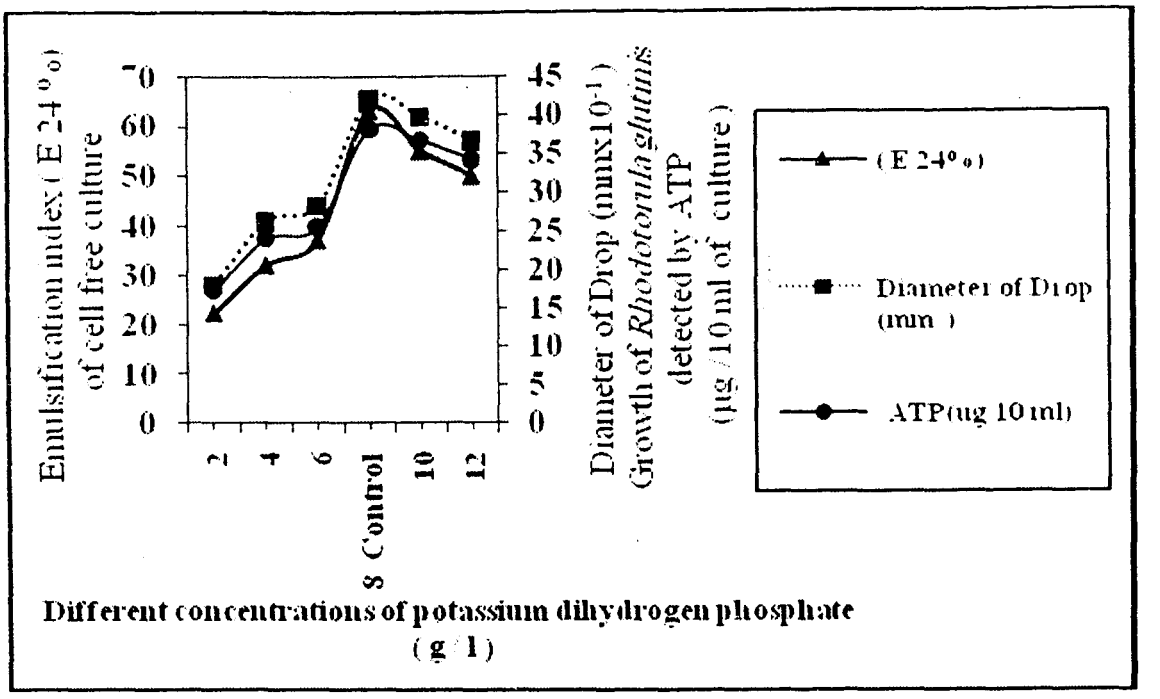

Fig. 7. Effect of different concentrations of potassium dihydrogen phosphate on the extracellular biosurfactant production and growth of *Rhodotorula glutinis*.

Concerning the effect of yeast extract, Cooper and Paddock (1983) mentioned that yeast extract is necessary for biosurfatctant production by *Torulopsis petrophilum*. Results in Fig. (8) Indicated that 1g % was the best concentration to increase the biosynthesis of extracelluar production of the biosurfactant .Similarly, Suehara *et al.* (2005) showed that 1g/l was optimal for diesel biodegradation by *Rhodotorula mucilaginous*.

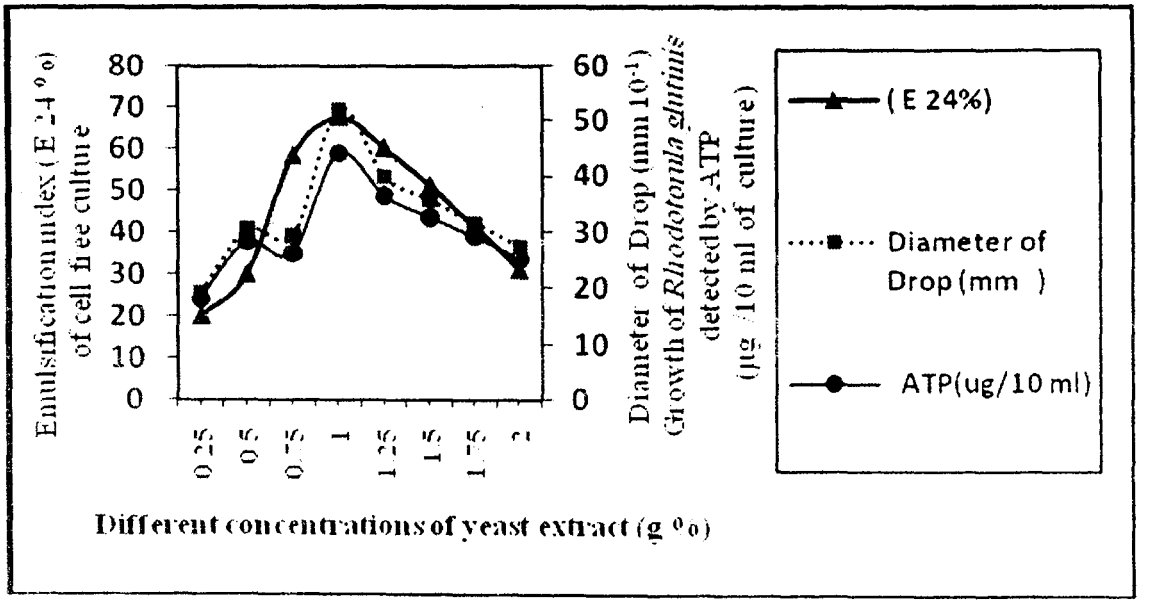

Fig. 8. Effect of different concentrations of yeast extract on the extracellular biosurfactant production and growth of *Rhodotorula glutinis*.

Concerning the effect of temperature and pH on both growth and extracelluar biosynthesis of emulsifier, results represented in Figs. (9 & 10) showed that the emulsifier activity was increased at 30°C and pH6, respectively. In this regard, the cell-free broth of *Candida lipolytica* was particularly influenced by the pH and temperature depending on the emulsified substrate (Sarubbo *et al.*, 2007). In the same regard, Kim *et al.* (2006) found that, the highest production yield of biosurfactant by *Candida* sp was obtained when the pH was controlled at 4.0, and the production was significantly improved, compared to batch fermentation without pH control.

In all investigated experiments with correllation coeffiecient at $p < 0.05$ indicated that the growth was increased with high significance parallel to the extracellular biosurfactant secretion. The total ATP was used as a good parameter for accurate estimation of the growth. In this respect, Forsberg and Lam (1977) stated that ATP is an easy quick and inexpensive for detection of growth.

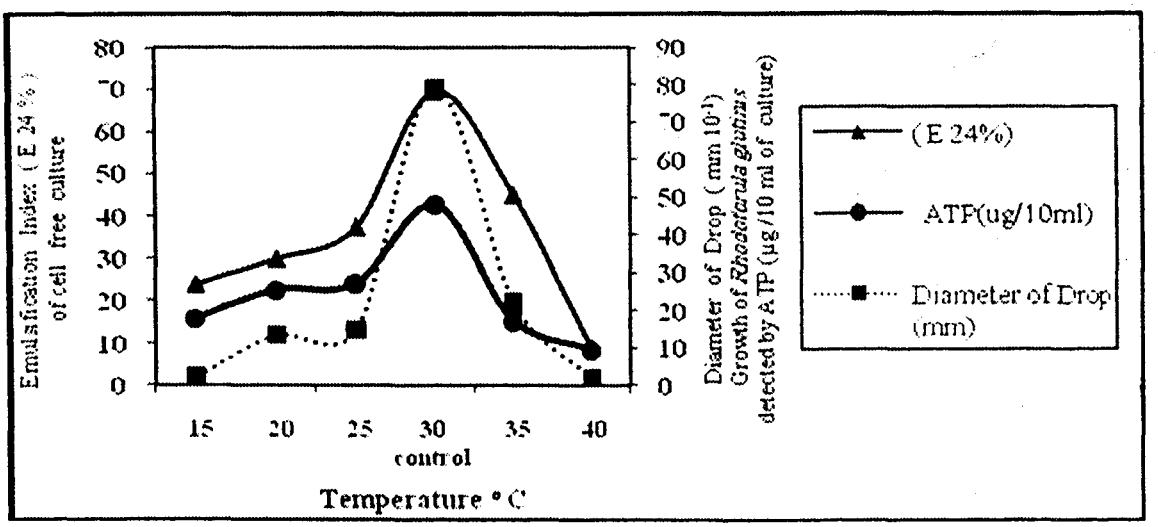

Fig. 9. Effect of different temperatures on the extracellular biosurfactant production and growth of *Rhodotorula glutinis*.

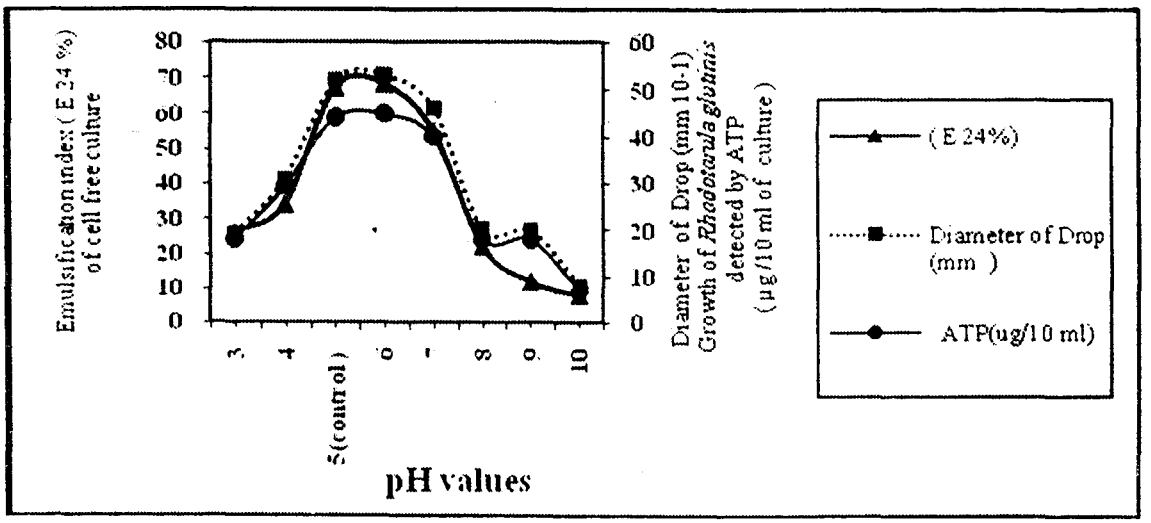

Fig.10. Effect of different pH values on the extracellular biosurfactant production and growth of *Rhodotorula glutinis*.

Purification and properties of the emulsions

Emulsifying activity was observed within the pH ranges from 2 to 10 and definite temperature from 20°C to -100°C, the maximum was obtained at pH 6 (Fig. 11) and 30°C (Fig. 12). The results showed that the biosurfactant had stable activity within a wide range of temperatures from 20 to 30°C, while the activity decreased from 40° to 100°C.

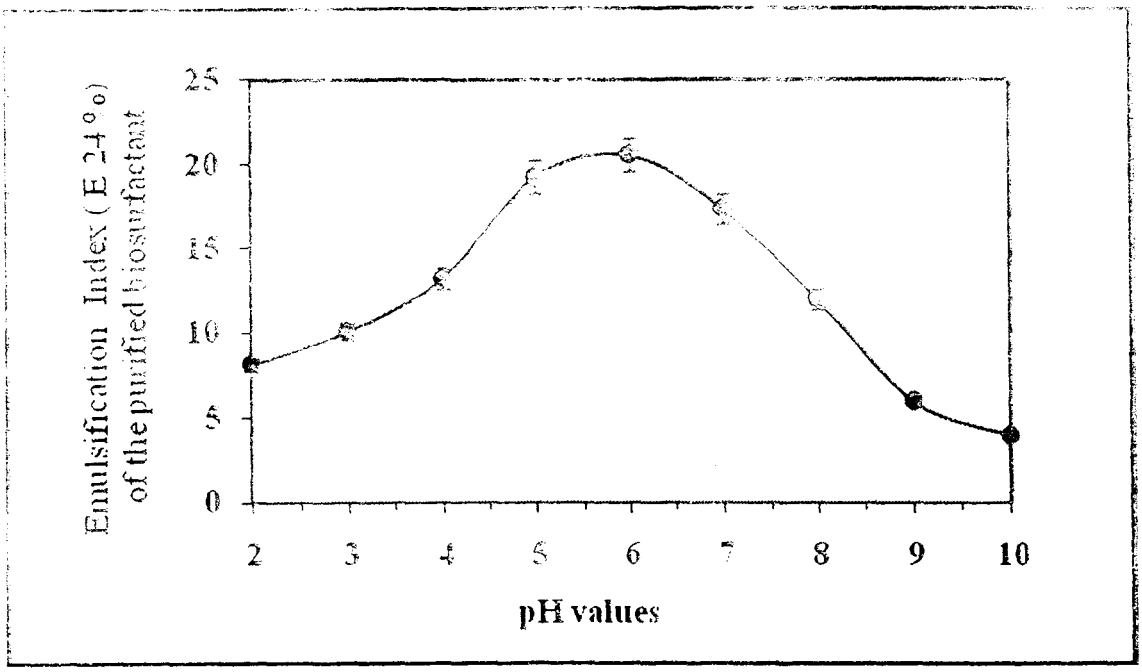

Fig.11. Effect of different pH values on the stability of the emulsifying activity of the purified biosurfactant.

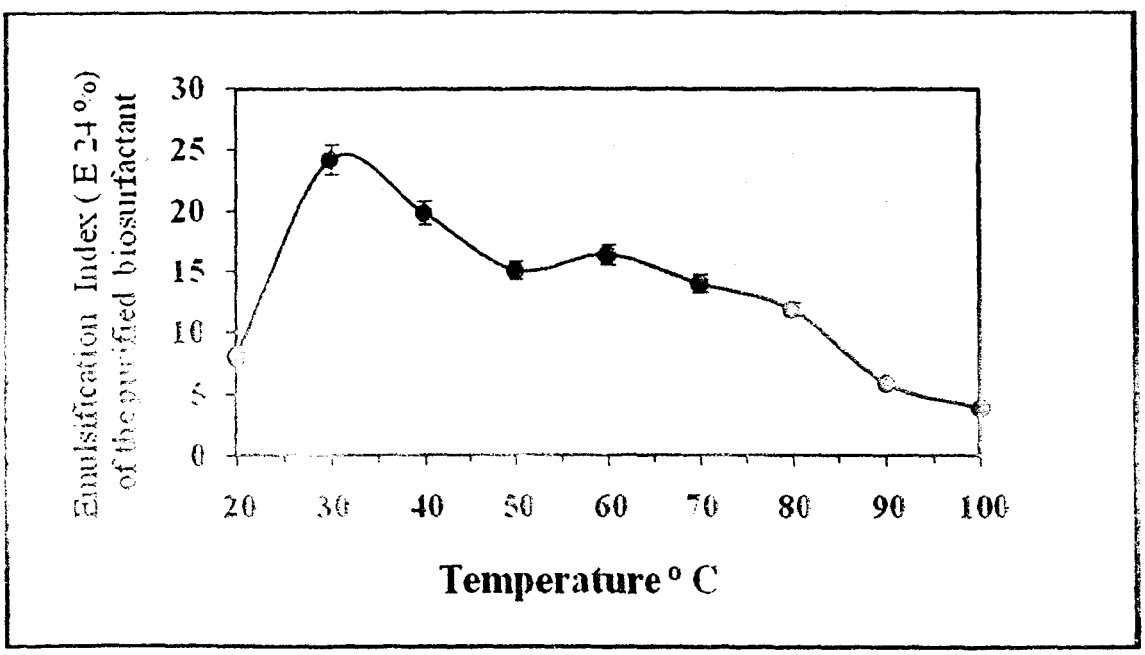

Fig. 12. Effect of different heat treatment on the stability of the emulsifying activity of the purified biosurfactant.

On the other hand biosurfactant activity was stable at pHs ranged from 2-10. In this respect, Navon –Venezia *et al.*, (1995) investigated the role of heat and pH on the stability of alas an which was isolated from *Acinetobacter radioresistens*. This property is important in the food and cosmetics industries, since it facilitate the formation of sterile, stable oil in water emulsion.

The analytical methods revealed that no lipids had been detected in the purified biosurfactant, while protein and carbohydrate were detected. Glycoprotein was detected in many types of yeast to form mat (Reynolds *et al.* 2007). Results in Table 2 showed the importance of the protein moieties in the biosurfactant activity in the purified polymer, since the apo-biosurfactant has no activity towards the recorded hydrocarbons. Maximum emulsifying activity was obtained with solar.

TABLE 2. Hydrocarbon substrate specificity of purified and Apo- biosurfactant bioemulsifer.

Hydrocarbon substrate	Emulsification index (E24%) of purified biosurfactant	Emulsification index (E24%) of Apo-biosurfactant
n-Heptane	0.16± 0.07	-
Toluene	1.11± 0.2	-
Sodium dodecyle sulphate	3.20± 0.4	-
Hexadecane	2.60± 0.2	-
Solar	14.12± 1.4	-

SDS-PAGE

The electrophoretic profile of different protein bands of the purified biosurfactant is represented in Table (3). Samples were treated at 100°C for 10 min in Laemmli buffer (Laemmli 1970) resolved by SDS-PAGE, and stained with Coomassie blue. The presented data revealed that the presence of 10 bands (MW:168,46 ; 36.491; 34.386; 29.583; 27.639; 25.139; 16.31; 11.286 and 8.1429 KDa).

Amino acid analysis

Amino acid analysis of hydrolyzed purified biosurfactant indicated the presence of three amino acids; alanine, methionine and proline (Table 4). In this regard, Navon - Venezia *et al.*, (1995) found that alanine was the major amino acid in alasan biosurfactant isolated from *Acinetobacter radioresistens.*

Sandpack test

Five concentrations of the purified biosurfactant were used to measure the least valuble concentration required for the most emulsification of sand contaminated oil. The results represented in Table 5 showed that the concentration 2% (w: v

biosurfactant : water) is the lowest concentration which gives a maximum emulsification activity. These results may reflect the ability of its use in a commercial scale. In this regard, Fiechter (1992) stated that although numerous biosurfactant have been discovered , but little of them was commercially applied due to their high cost.

TABLE 3. Electrophoretic protein profile of the purified biosurfactant.

Rows	Mol.Weight (KDa)	Mol.Weight Lane	Marker	Lane
r1				
r2	200			
r3	150	168.75		
r4	120			
r5	100			
r6	85			
r7	70			
r8	60			
r9	50	46.134		
r10	40			
r11		36.491		
r12		34.386		
r13				
r14	30	29.583		
r15		27.639		
r16	25	25.139		
r17	20			
r18		16.31		
r19	15			
r20				
r21	10	11.236		
r22		8.1429		

TABLE 4. Amino acid analysis of the purified emulsifier .

Amino acid analysis	Amino acid detected
Ala	+
Arg	-
Asp	-
Glu	-
Gly	-
His	-
Leu	-
Lys	-
Met	+
Phe	-
Pro	+
Ser	-
Thr	-
Tyr	-
Val	-

TABLE 5. Turbidity of the biosurfactant suspension measured by the optical density at 510 nm (suspension was diluted to x 10^{-3}) .

Different concentrations of the purified biosurfactant dissolved in dist.water % (w/ v biosurfactant : water)	Optical density of the diluted suspension
0.0 (control)	0.102
0.5	0.664
1.0	0.844
2.0	0.987
3.0	0.987
4.0	0.987

Conclusion

The results of study introduce a good hope to use this local cheap strain of yeast in elimination of solar from polluted systems. The ability of such strain was shown by either the consumption of solar as carbon source or by extracellular secretion of biosurfactant. The search for more strains need further studies, for their use in the elimination of mineral oil pollution in developing countries.

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إنتاج مخفض توتر سطحي كربوبروتيني من خميرة رودوتوريولا جلوتينيس المعزولة من تربة ملوثة بالسولار وإمكانية إستخدامه فى التخلص من التلوث بالسولار

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يعتبر التلوث بالزيوت المعدنية من المشاكل البيئية التى تمثل عبء إقتصادى خاصة فى الدول النامية نظرا لأرتفاع تكلفة المنظفات الكيميائية علاوة على تلويثها للبيئة ، ولقد زاد فى الأونة الأخيرة الأهتمام بأنتاج مخفضات التوتر السطحى الحيوية من الميكروبات حيث إنها تتميز بأمان أكثر وإتساع نطاق إستخدامها فى العديد من المجالات الزراعية والصناعية و الطبية المختلفة ، لهذا فقد إستهدفت الدراسة عزل سلالات خميرة قادرة علىإنتاج مخفضات توتر سطحى طبيعية حيث أفادت الدراسات السابقة قدرة بعض سلالات الخميرة على إستهلاك الزيوت المعدنية وكذا إنتاج مخفضات توتر سطحى تساعد على إذابة هذه الملوثات .

تم عزل سبع خمائر تابعة لخمس أجناس من خمسون عينة مختلفة تم تجميعها من أسفلت مرائب إيواء أتوبيسات النقل العام بالقاهرة . تم إختبار الرشيح الخلوى بعد التخلص من الخلايا لمعزولات الخميرة على منبت غذائى تخليقى يحتوى على السولار كمصدر كربونى للتحقق من قدرتها على الأفراز الخارجى لمخفضات التوتر السطحى ، تبين أن خميرة رودوتوريولا جلوتينيس أعطت أعلى نمو وأعلى إنتاج للمخفض ولهذا فقد أختبرت بعدد من العوامل الفيزيائية والكيميائية للوصول الى أعلى انتاج لمخفضات التوتر السطحى .

إستهدفت المرحلة التالية من الدراسة فصل كمية كبيرة من الرشيح الميكروبى وتنقيتها وتحليلها كيميائيا . تبين أن مخفض التوتر السطحى المفرز يتكون من بروتين ومواد كربوهيدراتية . أظهرت نتيجة فصل البروتين بتقنية التفريد الكهربى إحتواء البروتين على عشر حزم مختلفة الأوزان الجزيئية وبتحليل محتواها من الأحماض الأمينية تبين أن البروتين يتألف من ثلاث أنواع من الأحماض الأمينية، وباختبار قدرة البروتين على حده بعد فصله عن المحتوى الكربوهيدراتى فقد خاصيتة كمخفض للتوتر السطحى مما يثبت أهمية المحتوى الكربوهيدراتى فى البروتين المفصول .

فى دراسة أخيرة لأمكانية التطبيق العملى لمخفض التوتر السطحى على السولار تم أختباره بإستخدام تقنية المعجون الرملى والتى أثبتت أن ٢ % من المخفض ذائبا فى الماء يعمل على اذابة السولار .