Isolation and screening of biosurfactant producing yeast from soil contaminated with oil

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المستخلص:

هدف العمل الحالي الى عزل الخمائر من نماذج التربة الملوثة بالنفط ودراسة قدرتها على إنتاج المستحلبات الحياتية. جمعت خمسة نماذج من مواقع مختلفة في مدينة بغداد، تم الحصول على أربعة وثلاثون عزلة خمائر من نماذج التربة الملوثة . وجد ان تسعة عشر من هذه العزلات أبدت القابلية على إنتاج المستحلب الحياتي بالاعتماد على قطر المنطقة الشفافة في محلول الاختبار، بينما أعطت باقي العزلات نتيجة سلبية.
عزلات الخميرة التي أعطت نتيجة موجبة تم غربلتها كميا بالاعتماد على دليل الاستحلاب والشد السطحي، أظهرت الغربلة الثانوية إن العزلات SD5 وSB8 و SK10 أعطت الحاصل الأعلى من المستحلب الحياتي في مزارعها وان قيم دليل الاستحلاب لها كانت 45.7% و56.9% و 53.4% على التوالي. كذلك أعطى المستحلب الحياتي من العزلات SD5 وSB8 وSK10 القابلية الأكبر لخفض الشد السطحي للوسط إلى 38.2 و 27.1 و 30.8 mN\m على التوالي. العزلات المنتقاة SD5 وSB8 و SK10 تم تشخيصها بالاعتماد على الاختبارات المظهرية والكيموحيوية، أشارت النتائج من هذه الاختبارات إن العزلات تعود إلى *Saccharomyces cerevisiae* SB8 و *Candida glabrata* SD5 و *Candida glabrata* SK10 على التوالي.

اختبرت قابلية العزلات الثلاثة الاكفا على إنتاج المستحلب الحياتي في وسط BH المدعم بواسطة أربعة أنواع من المخلفات الزراعية (نخالة الرز، قشور الموز، نخالة الحنطة، عرانيس الذرة) كمصدر كاربوني مقارنة مع وسط BH المدعم بالكلوكوز. دلت النتائج ان جميع العزلات ابدت قابلية جيدة على انتاج المستحلب الحياتي في وسط BH السائل الحاوي على انواع مختلفة من المخلفات الزراعية وكذلك مع الكلوكوز، وان العزلة *Candida glabrata* SD5 اظهرت اعلى فعالية على انتاج المستحلب الحياتي عند تنميتها على المخلفات الزراعية والكلوكوز وبالاعتماد على قيم دليل الاستحلاب والشد السطحي، كما وان نخالة الحنطة كانت أفضل مادة أساس محفزة لإنتاج المستحلب الحياتي مقارنة مع بقية المخلفات الزراعية.

الكلمات الدالة: خمائر , تربة , مستحلب حياتي , فضلات زراعية .

Abstract:

The present study was aimed to isolate yeasts from soil samples contaminated with oil and their ability to produce biosurfactants. Five samples were collected from different sites of Baghdad, thirty four isolate of yeasts were obtained from these contaminated soil. Nineteen isolates from them were able to produce biosurfactant according to the diameter of clearing zonein solution test, while other isolates gave negative results. The yeast isolates which gave positive result werequantitatively screened on the basis of emulsification index and surface tension, secondary screening showed that isolates SD5, SB8 and SK10 giving the highest yield of biosurfactant in

their cultures and the values of emulsification index were 45.7%, 56.9% and 53.4% respectively. Also, the biosurfactant of isolates SD5, SB8 and SK10 were exhibited maximum ability to reduce the surface tension of medium to 38.2, 27.1 and 30.8 mN\m respectively. Finally selected isolates SD5, SB8 and SK10 were identification on the basis of morphological and biochemical tests, the results revealed that isolates were identified as *Saccharomyces cerevisiae*SB8, *Candida glabrata*SD5 and *Candida glabrata* SK10 respectively.The three most active isolates were tested to see their ability to produce biosurfactant in BH medium supplemented with four types of agricultural residues (rice bran, banana peel, wheat bran and corn cobs) as a source of carbon compared with BH medium with glucose. All isolates showed good ability to produce biosurfactant in liquid medium with different types of agricultural wastes and also with glucose. *Candida glabrata* SD5 exhibited highest activity for biosurfactant production when grown on agricultural wastes and glucose depend on the values of emulsification index and surface tension, whilethe wheat bran as a substrate gave best stimulation for biosurfactant production compared with other agricultural wastes.

Key words: Yeast, Soil, Biosurfactent ,Agricultural wastes.

have a wide range of industrial application including pharmaceutics, medicine, agricultural, food, cosmetics industries it was petroleum [8].Besides being useful in bioremediation and improved degradation of chemical contaminants in contaminated sites [9].Biosurfactants produced from various substrates including sugar,glycerol, oil, hydrocarbons and agricultural wastes[10].The most promising substrate for low cost biosurfactant production are agro-industrial wastes which can reduce many processing industrial wastes management problems [11].This would lead to reduced pollution caused by those wastes and economical biosurfactant production [12].

The most important biosurfactant producing microorganisms reported from the species like *Bacillus* sp., *pseudomonas* sp. and *candida* sp. which known to produce lipopeptide,rhamnolipids and glycolipids surfactants[13].The possibility of increasing the range of uses for biosurfactant obtained from yeast is due to their GRAS (generally

Introduction:

Surfactant active compounds are synthetic compounds that produced by chemical reaction [1]. These compounds can be derived naturally byvariety of yeast, bacteria and filamentous fungi [2]. The production of these compounds has become more important in recent years [3].It is due to their different advantages such as higher biodergradability, higher selectivity, higher foaming,better environmental capability, metal binding,specific activity at extreme temperature,pH and salinity and the ability to be synthesized from renewable feed stocks [4;5].Besides being less toxic than chemical surfactant [6].

Biosurfactant are categorized into the following groups; glycolipids, lipopeptides, phospholipide , fatty acid salt and polymeric biosurfactant.These compounds reduce surface and interfacial tensions by accumulation at the interface between two fluids such as oil and water [7]. Biosurfactant

incubated at 30 °C in a rotary shaker at 150 rpm for 7days, after incubation period the cultures were centrifuged at 8000 rpm for 20 min. and the supernatants were used to detect the primary ability of yeast isolates to produce biosurfactant using oil-spreading method, then secondary screening was achieved for isolates that gave positive result in primary screening using two different techniques (emulsification index andsurface tension) as below:

Oil-spreading method

50 ml of distilled water was poured into theplates, and then 50 ml of crude oilwas added on the surface of water to generate thin film covered the surface of water. Then ,10 ml from supernatants of each yeast isolate was added into center of plates. Thediameter of clear zone observed on the surface was measured [21].

Emulsification index method

The emulsification ability of the biosurfactant produced in yeast cultures were studied by adding 1 ml of diesel oil to tubes containing1 ml of crude biosurfactant, then mixing for 2 min by vortex and left it stand for 24 h. The emulsification index (E24)as percentage was calculated using below equation [22].

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

Surface tension method

The surface tension for cell free supernatants of yeast cultures and positive controls were measured by digital Tensiometer using Du Ring method at 25± 1 ºC[23].

Identification of yeasts

The identification of three selected yeasts (SD5, SB8 and SK10) were diagnosed based on cultural characteristic (such as gram staining, color, texture, shape and surface appearance), regarded as safe) which means no risk of toxicity and pathogenic reaction [14]. *Candida* species have been frequently reported as producers of emulsifying agent [15]. Glycolipid biosurfactant produced by various yeast strain one of them is *Candida batistae*[16].Sophorolipids biosurfactant are produced mainly by yeast ,such as *Candida apicola*and *Candida bombicola*[17]. The production of biosurfactant occurs by *Candida glabrata* are demonstrated by [18].

Materials and Methods

Samples collection and yeast isolation

Five samples of soil contaminated with oil were collected during the period of March-April 2017 from different sites at Baghdad city. 1 g from each sample was transferred to a glass test tube containing 9 ml of distilled water and shaken vigorously, then serial dilutions were prepared. 100 µl from serial dilutions (10^{-3}-10^{-5}) were transferred to plates of sterile Sabouraud dextrose agar (SDA) supplemented with chloaramphenicol (250 mg/l) and spread into the surface of sterile plates.

The plates were incubated at 30°C for 3-7 days. Single colonies of yeast isolates were selected and purified by streaking them on the same medium. The pure cultures were transferred into slants of SDA and incubated at 30 °C for 2-3 days and stored in the refrigerator at 4 °C.[19].

Screening for Biosurfactant production

Pure isolates of the yeast were screened for biosurfactant production using Bushnell-Haas medium containing (g\l):K_2HPO_4 (1), KH_2PO_4 (1),NH_4NO_3 (1), $MgSO_4 \cdot 7H_2O$ (0.2),$CaCl_2$ (0.02) and$FeCl_2$ (0.05), the medium was supplemented with 2% glucose and 0.1%yeast extractas carbon and nitrogen sources[20].

The pure yeast isolates were inoculated in flasks of liquidBushnell-Haas medium, pH 7and

Table (1): yeasts isolation from soil samples contaminated with oil that collected from different sites of Baghdad, using Sabouraud dextrose agar plates.

Soil samples	Symbol	Collection period	Number of isolates
Al-Dora	SD	5th March, 2017	7
Al-Shuala	SS	16th March, 2017	5
Al-Bayaa	SB	27th March, 2017	9
Al-Kadimia	SK	9th April, 2017	10
Al-Jadriya	SJ	23th April, 2017	3
Total isolates = 34			

Biosurfactant producing microorganisms were naturally present in oil contaminated soil [26]. Because they are adapted to grow in environments contaminated with these compounds[27].Study by [28] isolated 11 different yeast strains from three sites contaminated with products of refined petroleum and oily residue using sabouraud dextrose agar (SDA) supplemented with chloramphenicol at concentration of 200 mg/l at 28 °C for up to 10 days. Also,[29] reported the isolation of yeast isolate from oil- contaminated soil using modified mineral salt medium supplemented with steam sterilized diesel oil ,sodium propionate and chloramphenicol at 30 °C for 5 days. So,[30] isolated a total of 8 fungal isolates from soil samples contaminated with engine oil using mineral salt medium and sabouraud dextrose agar at 28°C.

Screening of yeast isolates

The ability of yeast isolates to produce biosurfactants in liquid Bushnell-Haas medium was detected by oil-spreading method as easy and primary step. Results in table (2) showed that only nineteen isolates were appeared the diameter of clearing zone in solution test, while other isolates gave negative results. Additionally the results revealed that the isolates SD5, SS3, SB4, SB8 and SK10 formed the highest diameter of clearing zone than other isolates.

morphological propertiesand biochemical tests[24].

Biosurfactant production using waste

Different renewable wastes (rice bran, banana peel, wheat bran and corn cobs) were collected from different sites. Then the wastes were dried and milled to a particle size less than 1 mm to use as sources of carbon in medium forbiosurfactant production.

The glucose in Bushnell-Haas medium was replaced with 2% of agriculture wastes (rice bran, banana peel, wheat bran and corn cobs)to detect the ability of most active isolates SD5, SB8 and SK10 to produce biosurfactant. The flasks were inoculated and incubated at 30 ^{o}C in shaker incubator at 150 rpm for 7days, after incubation period the cultures were centrifuged at 8000 rpm for 20 min. and the supernatants were used to measure the ability of most active isolates to produce biosurfactant using tests of emulsification index and surface tension as above methods. The flasks of Bushnell-Haas medium with 2% glucose were used as positive control in current experiment[25].

Results and Discussions

Sampling and yeasts Isolation

Thirty four isolate of yeasts were obtained from five samples of soil contaminated with oil waste that collected from different sites in Baghdad city using Sabouraud dextrose agar plates as in(table 1).

Moreover, the results showed differences in the numbers of yeast isolates that isolated from different soil samples. These differences may be occured due to nature of sites and the period of their contamination with hydrocarbons.

Table (3): Secondary screening of yeast isolates by emulsification index method, the isolates were inoculated in liquid BH medium, pH 7 with 2% glucose and incubated for 7 days at 30 ◦C.

Isolates	Emulsification index (%)	Isolates	Emulsification index (%)
SD1	26.7	SB7	36.6
SD3	33.6	SB8	56.9
SD5	45.7	SK1	22.4
SD7	35.9	SK3	32.9
SS3	40.2	SK5	19.5
SS5	26.8	SK7	29.1
SB2	34.2	SK8	36.5
SB3	37.1	SK10	53.4
SB4	41.9	SJ1	33.8
SB5	28.7		

Table (4): Secondary screening of yeast isolates by surface tension method, the isolates were inoculated in liquid BH medium, pH 7 with 2% glucose and incubated for 7 days at 30 ◦C.

Isolates	Surface tension (mN/m)	Isolates	Surface tension (mN/m)
SD1	59.1	SB7	45.9
SD3	42.6	SB8	27.1
SD5	38.2	SK1	55.4
SD7	44.2	SK3	43.7
SS3	40.3	SK5	58.4
SS5	60.2	SK7	56.9
SB2	44.1	SK8	42.4
SB3	46.8	SK10	30.8
SB4	40.6	SJ1	46.7
SB5	58.1		

Oil spreading technique was used to measure the diameter of clear zone caused when a drop of a biosurfactant containing placed on oil – water surface [31].Oil spreading method was used by [32] for screening of biosurfactant from yeast and bacterial isolates , results showed that the production of clear zone in the maximum level by *Candida tropicalis*, *Candida albicans* and *P. aeroginosa* . Also,[33] reported that oil spreading test was better in detection of small quantity of biosurfactant.The diameter of the clear zone on the oil surface correlated to surface activity [34]. A large diameter of clear zone represents a higher activity of biosurfactant[35].

Table(2): Primary screening of yeast isolates by oil-spreading method, the isolates inoculated in liquid BH medium, pH 7 with 2% glucose and incubated for 7 days at 30 ◦C.

Isolates	Diameter of clear zone	Isolates	Diameter of clear zone	Isolates	Diameter of clear zone
SD1	B	SB1	A	SK4	A
SD2	A	SB2	C	SK5	B
SD3	C	SB3	C	SK6	A
SD4	A	SB4	D	SK7	B
SD5	D	SB5	B	SK8	C
SD6	A	SB6	A	SK9	A
SD7	C	SB7	C	SK10	D
SS1	A	SB8	D	SJ1	C
SS2	A	SB9	A	SJ2	A
SS3	D	SK1	B	SJ3	A
SS4	A	SK2	A		
SS5	B	SK3	C		

* no clear zone(A), less than 1 cm(B), from 1-3 cm(C), more than 3 cm(D)

The secondary screening to produce biosurfactant was achieved for nineteen isolates that gave positive results in primary detection. The free-cell cultures of yeast isolates were used in this step using the methods of emulsification index and surface tension as indicators.

The results in table (3) showed a variation in ability of isolates to produce biosurfactant and the isolates SD5, SB8 and SK10 were exhibited the best ability to release thebiosurfactant in liquid cultures than other isolates. Where the maximal emulsification index (%) with kerosene were found to be 45.7%, 56.9% and 53.4% respectively.

Also, the ability of isolates to reduce the surface tension of free-cell cultures was demonstrated. As shown in table (4) the results confirmed that the isolates SD5, SB8 and SK10 gives the highest ability to decrease the surface tension of supernatants from 70mN\m for control to 38.2 , 27.1 and 30.8mN\m for isolates respectively.

polluted soil [44]. Microscopic examination and biochemical tests were used to identified the higher biosurfactant producers yeast isolates from soil samples collected from polluted location by[45],results showed that these isolates might be identified as *Candida spp.*,*Saccharomycopsis spp.* and*Brettanomyces spp*.

Production of biosurfactant using wastes

The low-cost carbon sources (rice bran, banana peel, wheat bran and corn cobs) were used as substrates to produce economical biosurfactant using liquid BH medium supplemented with 2% from each agricultural waste and inoculated with most active isolates SD5, SB8 and SK10 for 7 days at 30 ^{o}C.

The table (5) shows the results of emulsification index (%) and surface tension for test and positive control flasks, andthe values of emulsification indexand surface tension as in table (5) provided that all studied isolates have good ability to produce the biosurfactant inliquid media supplemented with rice bran, banana peel, wheat bran and corn cobs.

Also the results revealed that the wheat bran and rice bran gives the best production than other substrates and the medium supplemented with wheat bran and inoculated with *Candida glabrata* SD5 gave the best results forbiosurfactant production compared withother isolates and positive control.

Most researchers have used a maximum of two to three screening methods before selecting biosurfactant producers [23]. [36] investigated the production of biosurfactant by primary screening for selected yeast isolates by using fermentative medium. Emulsification index is considered reliable accurate method to screen isolates having capability to produce biosurfactant[37].Biosurfactant production by yeast isolate was confirmed by its emulsification index, the obtained results appeared that yeast L 69 showed 50% emulsification index in engine oil and soy-bean,while L 104 showed 93% of emulsification index in engine oil and soy- bean [38]. [39]reported emulsification index of 44% against kerosene of biosurfactant produced by the isolate (H11) isolated from contaminated soil with oily wastes .

Moreover, the Biosurfactant reduce the surface tension of both aqueous solutions and hydrocarbon mixtures [40].A good Candidate for biosurfactant production should reduce the surface tension of the medium to lower than 40 mN/m[41]. The most effective biosurfactant can reduce the surface tension of water from 72 mN/m to values in the range of 25-30 mN/m[42].The biosurfactant produced by yeast isolateWG1 showed a significant reduction in surface tension ofcell free suspension CFS from 72 to 39.2mN/m.[43].

Identification of yeast isolates

The three most active isolates from secondary screening were identified on the basis of some morphological and biochemical tests, the results from these tests shown that the three isolates SD5, SB8 and SK10 belong to *Saccharomyces cerevisiae*SB8, *Candida glabrata* SD5 and *Candida glabrata* SK10 respectively.

Previous studies mentioned the isolation of some similar genera encountered in this study such as *Candida*,*Saccharomyces* and *Cryptococcus* from three different crude oil

Mecheri et al., [50] reported that isolation of yeast isolate from hydrocarbons contaminated area , the obtained yeast isolate was the most potent biosurfactant yeast with an emulsification index of 69.50 and emulsion over than 60% and have the ability to produce biosurfactant when grown in low –cost fermentative media based on agro industrial by –products . Biosurfactant production by yeast isolate using low-cost agro industrial wastes were investigated by [51],mineral salt medium supplemented with corn steep liquor CSL (20%) and whey WH (40%)from diary industry and inoculated with 5% of *Candida glabrata*at 28 °C and 150 rpm ,the obtained biosurfactant reduced the surface tension to 28.8 mN/m after 72h. Among the yeast ,*Candida glabrata*has been reported as microorganism with high potential for producing biosurfactant from agro-industrial wastes [52 ; 53].

Table (5): Effect of different carbon sources on biosurfactant production by isolates SD5, SB8 and SK10 using BH medium, flasks incubated at 30°Cfor 7 days.

Agricultural wastes	Emulsification index (%)			Surface tension (mN\m)		
	SD5	SB8	SK10	SD5	SB8	SK10
Rice bran	39.3	45.6	43.5	44.7	40.6	42.1
Banana peel	31.2	33.4	32.8	51.8	47.2	49.1
Wheat bran	42.6	47.2	45.3	42.1	38.1	40.5
Corn cobs	26.8	29.6	29.1	54.5	51.9	52.6
Glucose	44.7	56.9	53.4	38.2	27.1	30.8

Biosurfactant are produced by bacteria or yeast from various substrates including sugar,glycerol, oils, hydrocarbons and agricultural wastes [46]. Agro-industrial wastes are considered as the most promising substrate for low coast biosurfactant production [47].Similarly, seven yeast isolates were isolated from soil contaminated with petroleum oil hydrocarbon showed the ability to produce biosurfactant in low cost substrate (soybean oil),the isolate LBPF3 which characterized as *Candida Antarctica* showed highest level of production 13.86 g/l and highest reduction in surface tension in the growth medium 43% after 24 h of incubation [36]. Reduction in surface tension of media from 30mN/mto minimum values of 26mN/m was observed by[48] who studied the production of biosurfacrant by *Candida sphaerica*using soapstock as a source of carbon. Also,[49] studied the production of biosurfactant by different species of yeast using low cost substrate, the obtained results verified the reduction in surface tension in media from 57mN/m to a values varying from 35mN/m to 40mN/m.[18] found that biosurfactant produced by *Candida glabrata*using glucose plus cotton seed oil ,lower the surface tension from 68mN/m to 31mN/m .

References:

1. **Jaysree, R.C., Basu, S., Singh, P.P., Ghosal, T., Patra, A.P., Keerthi, Y. and Rajendran, N. (2011).** Isolation of biosurfactant producing bacteria from environmental samples. *Pharmacologlyonline*3:1427-1433.
2. **Anandaraj, B. &Thivakaran, P. (2010).** Isolation and production of biosurfactant producing organism from oil spilled soil. Biosci Tech,1(3): 120-126.
3. **GhayyomiJazeh, M., F. Forghani and Deog-Hwan Oh.(2012).**Biosurfactan Production by Bacillus sp.Isolated from Petroleum Contaminated Soils of Sirri Island. American Journal of Applied Sciences 9 (1): 1-6.

for EnhancedOilRecovery . Biotechnology and Bioengineering, 98(4): 842-853.

12. **Maneerat, S., T. Bamba, K. Harada, A. Kobayashi, H. Yamada and F. Kawai.(2006).** A novel crude oil emulsifier secreted in the culture supernatant of a marine bacterium, Myroides sp. strain SM1. Appl. Microbiol. Biotechnol., 70: 254–259.
13. **Rahman, P.K.S.M.; and Gakpe, E. (2008).** Production, characterisation and applications of biosurfactant- Review. Biotechnology, 7(2): 360-370.
14. **Fontes, G. C.; Amaral, P. F. F. E.and Coelho, M, A, Z. (2008).**Produção de biossurfactanteporlevedura. Quimica Nova. 40: 316-323.
15. **Rufino, R.D.; Sarubbo, L.A.; Campos-Takaki.; G.M.J. (2007).** Enhancement of stability of biosurfactant produced by *Candida lipolytica* using industrial residue as substrate. W. J. Microbiol. Biotechnol. 23: 729-734.
16. **Van Bogaert, I. N.; Saerens, K.; De Muynck, C.; Develter, D.; Soetaert, W. and Vandamme, E.J.(2007).** Microbial production and application of sophorolipids. ApplMicrobiolBiotechnol.;76:23–34.
17. **Konishi, M.; Morita T., Fukuoka, T.; Imura T.; Kitamoto, D. (2008).** Production of new types of sophorolipids by *Candida batistae*, J. Oleo Sci.. 57: 359–369.
18. **Sarubbo, L.A.; Luna.; J.M. ; Campos-Takaki,G.M. (2006).** Production and stability studies of the bioemulsifier obtained from a new strain of Candida glabrata UCP 1002 .Electron. J. Biotechnol. 54: 68-73.
19. **Goulart GG, Coutinho JOPA, Monteiro AS, Siqueira EP and Santos VL(2014).**Isolation and Characterization

4. **Chaprão,M.J.; Isabela N.S. Ferreira, Priscilla F. Correa, Raquel D. Rufino, Juliana M. Luna,Elias J. Silva, Leonie A. Sarubbo.(2015).** Application of bacterial and yeast biosurfactants for enhanced removal and biodegradation of motor oil from contaminated sand. Electronic Journal of Biotechnology 18 : 471–479.
5. **Silva, R.C, Almeida, D.G, Rufino, R.D, Luna, J.M, Santos, V.A, and Sarubbo, L.A.(2014).** Applications of biosurfactants in the petroleum industry and the remediation of oil spills. Int J MolSci, 15(125): 23–42.
6. **Marajan ,C.; Z. MohdZaki, Z.; Ramasamy, K. and Abdul-Talibs, S. (2015).I**dentification and screening charachterisation of potential biosurfactant producing bacteria isolated from palm oil mill effluent. Journal of Engineering Science and Technology.8: 94 – 102.
7. **Banat, I.M., Franzetti, A., Gandolfi, I. (2010).** Microbial biosurfactants production, applications and future potential. Appl. Microbiol. Biotechnol., 87: 427–444.
8. **Kamal-Alahmad. (2015).**The Definition, Preparation and Application of Rhamnolipids as Biosurfactants. International Journal of Nutrition and Food Sciences, 4(6): 613-623.
9. **Van Hamme, J.D., Singh, A. & Ward, O. (2006).** Physiological aspects Part 1 in a series of papers devoted to surfactants in microbiology and biotechnology. Biotechnology Advances, 24: 604–620.
10. **Lin, SC. (1996).** Biosurfactant: Recent advances. J ChemTechnolBiotechnol 63:109–120.
11. **Wang,Q.; Fang,X.; Bai,B.; Liang,X.; Shuler,P.J.; Goddard III,W.A. and Tang,Y.(2007).** Engineering Bacteria for Production of Rhamnolipid as an Agent

27. **Kilbane, J.J. ;Ranganathan, R.; Cleveland, L.; Kayser, K.J.andRibiero, C. (2000).** Selective removal of nitrogen from quinoline and petroleum by Pseudomonas ayucida IGTN9m. Appl Environ Microbiol 66: 688-693.
28. **Goulart, G.G., Coutinho, J.O.P.A.; Monteiro, A.S.; Siqueira, E.P. and Santos, V.L.(2014).** Isolation and Characterization of Gasoline-Degrading Yeasts from Refined Oil-Contaminated Residues. J BioremedBiodeg. 5(2): 1-9.
29. **Yao Shumin; Ni Na; XuZhaoyanga and Tan Jinglia.(2012).** Screening and identification of halotolerant yeast for hydrocarbon degrading and its properties studies. Afr. J. Microbiol. Res., 6(8): 1819-1828.
30. **Mbachu, A.E.;Chukwura, E.I. and Mbachu, N.A .(2016).** Isolation and Characterization of Hydrocarbon Degrading Fungi from Used (Spent) Engine Oil Polluted Soil and Their Use for Polycyclic Aromatic Hydrocarbons (PAHs) Degradation. Universal Journal of Microbiology Research 4(1): 31-37.
31. **Morikawa, M., Hirata,Y. and Imanaka,T.(2000).** A study on the structure function relationship of the lipopeptidebiosurfactants. Biochimica et Biophysica .Acta, 1488: 211-218.
32. **Padmapriya,B.; Suganthi,S. and Anishya,R.S.(2013).** Screening, Optimization and Production of BiosurfactantsbyCandida Species Isolated from Oil Polluted Soils. J. Agric. & Environ. Sci., 13 (2): 227-233.
33. **Hamzah, A.; Sabturani, N. and Radiman, S.(2013).**Screening and optimization of biosurfactant production by the hydrocarbon-degrading bacteria. SainsMalaysiana, 42(5): 615–623.
34. **Diab,E.A.(2013).** Screening Bacterial Strains Isolated from Used Motor oil-of Gasoline-Degrading Yeasts from Refined Oil-Contaminated Residues. J. Bioremed. Biodeg., 5 (2): 1-9.
20. **Chandran, P. and Das, N. (2010)**.Biosurfactant production and diesel oil degradation by yeast species *Trichosporonasahii*isolated from petroleum hydrocarbon contaminated soil. Int. J. Eng. Sci. Technol., 2: 6942–6953.
21. **Morikawa M, Hirata Y, Imanaka T,(2000)**. A Study on the structure-function relationship of lipopeptidesbiosurfactants. BiochimicaetBiophysicaActa. 1488: 211-218.
22. **Cai, Q., Zhang, B., Chen, B., Zhu, Z., Lin, W., Cao, T., (2014)**. Screening of biosurfactant producers from petroleum hydrocarbon contaminated sources in cold marine environments. Marine pollution bulletin, 86: 402-410.
23. **Youssef, N., Duncan, K. and Nagle, D. (2004).** Comparison of methods to detect biosurfactant production by diverse microorganisms. J. Microbiolog. Metho. 56(3): 339-347.
24. **Yarrow, D. (1998)**. Methods for isolation, maintenance and identification yeasts. In-Kurtzman CP, Fell J (editors): The Yeasts - a taxonomic study. Elsevier Science Pub., Netherlands.
25. **Jimoh S. O., Adefioye N. A., Bakare R. I., Ibrahim R. A. and Ashorobi A. A. (2015)**. Physicochemical screening of *Candida lusitaniae*P1 during synthesis of biosurfactant from coconut shell. Malaysian J. Microbiol., 11(3): 306-312.
26. **Femi-Ola, T. O., Oluwole, O. A., Olowomofe, T. O. and Yakubu, H.(2015).** Isolution and screening of biosurfactant-producing bacteria from soil contaminated with domestic waste water. British Journal of Environmental Sciences.l.3(1): 58-63.

Somboonthanate, P. and Chavadej, S. (2010). Biosurfactant production by Pseudomonas aeruginosa SP4 using sequencing batch reactors: effect of oil-to-glucose ratio. Biochemical Engineering Journal. 49: 185-191.

42. **Agarwal, P.; Sharma, D.K. (2009).**Studies on the production of biosurfactant for the Microbial Enhanced Oil Recovery by using bacteria isolated from oil contaminated wet soil. Pet Sci Technol. 27:1880–1893.
43. **Vidur, B..andBaljeet, S. S. (2016).**Isolation and partial Structural & Functional Characterization of Glycolipid Biosurfactant producing Pichiasorbitophila WG1 from Rotten Grapes. 8(7):357-367.
44. **Wemedo S.A. and Awah V.W.(2017).**Occurrence of Yeasts in an Experimental Crude Oil Contaminated Soil in Nigeria. Journal of Biology and Genetic Research, 3(1): 20-26.
45. **Kaur, K.; Seema, S. and Harpreet, K.(2017).**Biosurfactant production by yeast isolated from hydrocarbon polluted environment.12: 189:603.
46. **Lin, S.C. (1996).**Biosurfactants: recent advances. J. Chem. Tech. Biotechnol. 66:109-120.
47. **Maneerat, S. (2005).** Production of biosurfactants using substrates from renewable resources. Songklanakarin J SciTechnol 27(3):675– 683.
48. **Souza- Sobrinho, H.B. (2007).**Utilização de resíduosindustriaiscomosubstratos de baixocustopara a produção de biossurfactantepor Candida sphaerica .Pernambuco, Brasil, 99p. (M.Sc. Dissertation. UniversidadeCatólica de Pernambuco).
49. **Haba, E.; Espuny, M.J.; Busquets, M.and Manresa, A. J. (2000).** Screening and production of rhamnolipids by Pseudomonas aeruginosa 47T2 NCIB Polluted Desert Soil for the production of Biosurfactants and the Possibility of Applying the Produced Biosurfactants for Washing and Bioremediation of the Polluted Soil. IJSR, 4(8):887-895.
35. **Rodrigues, L.R., Teixeri, J. A., Van der Mei, H.C., Oliverra, R. (2006).** Physicochemical and functional characterization of a biosurfactant produced by Lactococcuslactis 53. Colloids Surf B Biointerfaces 49:79-86.
36. **Accorsini,F.R.; Mutton2,M.J.R.; Lemos,E.G.M. and Benincasa,M.(2012).** Biosurfactant production by yeasts using soybean oil and glycerol as low cost substrate. Brazilian Journal of Microbiology, 116-125.
37. **Ahmad, Z., M. Arshad, H.N. Asghar, M.A. Sheikh and D.E. Crowley.(2016).** Isolation, screening and functional characterization of biosurfactant producing bacteria isolated from crude oil contaminated site. Int. J. Agric. Biol., 18: 542–548.
38. **Sousa, T.; Pinheiro, T.; Coelho, D.;Tambourgi, E.B.; Sette, L.;Pessoa, Jr. A.; Cardoso, V.; Silveira, E.andCoutinho-Filho, U.(2016).** Evaluation of biosurfactant production by yeasts from antarctica, Chemical Engineering Transactions, 49, 547-552.
39. **Velmurugan, M., et al. (2015).** Screening, stability and antibacterial potential of rhamnolipids from Pseudomonas sp., isolated from hydrocarbon contaminated soil. Journal of Applied Pharmaceutical Science, 5(08), 026-033.
40. **Pradnya, A. J. and Dhiraj B. S.(2014).** Screening and isolation of biosurfactant producing bacteria from petroleum contaminated soil. Euro. J. Exp. Bio., 4(4):164-169.
41. **Pansiripat, S., Pornsunthorntaweea, O., Rujiravanit, R., Kitiyanana, B.,**

40044 from waste frying oils. Appl. Microbiol. 88, 379–387.

50. **Mecheri, L.D.; GanaS.K. ;TalbiS.K.andDjenane D.(2017)**. Screening and biosurfactant/bioemulsifier production from a high-salt-tolerant halophilic Cryptococcus strain YLF isolated from crude oil. Elsevier B.V. 1 November.
51. **Lima,R.A.; Andrade,R.F.S.; Rodríguez,D.M.; Araújo,H.W.C.; Santos,V.P. and Campos-Takaki,G.M..(2017).** Production and characterization of biosurfactant isolated from Candida glabrata using renewable substrates. Afr. J. Microbiol. Res., 11(6): 237-244.
52. **Andrade RF, Antunes AA, Lima RA, Araújo HW, Resende-Stoianoff MA, Franco LO, Campos-Takaki GM (2015).** Enhanced Production of an Glycolipid Biosurfactant Produced by Candida glabrata UCP/WFCC1556 for Application in Dispersion and Removal of Petroderivatives. Int. J. Curr. Microbiol. Appl. Sci., 4(7):563-576.
53. **Nhlanhla, T. ;Patel, M. and Francis, D.M. (2016).** Examination of Candida albicans strains from South Africa for the production of gliotoxin and other cytotoxic secondary metabolites. J. Yeast Fungal Res., 7(3):19-27.