Al-Kufa University Journal for Biology / VOL.9 / NO.3 / Year: 2017

Print ISSN: 2073-8854 & Online ISSN: 2311-6544

# Isolation of biosurfactant/bioemulsifier from *Saccharomyces cerevisiae* by different treatment

**Duaa Haider Raham** **Nibras Nazar Mahmood**

## Abstract

Biosurfactant are amphiphilic compound have been increasingly attracting the attention of the scientific community as promising candidates for the replacement of a number of synthetic surfactants. The aim of this study is to extraction of Biosurfactant from *Saccharomyces cerevisiae* by using three treatment (absolute ethanol , ammonium sulphate and heating). The present study clearly showed that , the treatment with boiling to 30 min was very effect to extract the biosurfactant from *S. cerevisiae* best than other treatments with high emulsification index and surface activity where were 81.08% and 20 mm , respectively.

**Key words** : Biosurfactant , Bioemulsifier , Saccharomyces cerevisiae

## Introduction

Microorganisms have ability to produce a broad range of molecules. amongst them there are amphipathic molecules, known biosurfactants (Fracchia et al., 2012; Willem , 2012).

Surfactants are generally organic compounds that are amphiphilic, , concept they contain both hydrophobic groups (‘‘tails’’) and hydrophilic groups (‘‘heads’’), and that act preferably in the interface of fluid phases with various levels of polarity and bridges of hydrogen, like oil/water or air/water Interfaces (Luna *et al*., 2011). Biosurfactants were created by different groups of microorganisms (Sridhar *et al*., 2013). Due to their good properties like lower toxicity, higher foaming capacity , higher biodegradability and higher activity at extreme temperatures,salinity and pH levels (Prabakaran and Sumathi, 2014). biosurfactants have been increasingly attracting the interest of the scientific community as promising candidates for the instead of a number of synthetic surfactants (Vidhya *et al*., 2015). These compounds are biological molecules with remarkable surfactant characteristics identical to the well‑known synthetic surfactants and they also include microbial compounds with surfactant characteristics (Trindade *et al*., 2008). the present study has carried out to test absolute ethanol , ammonium sulphate and heating treatments to obtain of biosurfactant/bioemulsifier from *S. cerevisiae* with high emulsification activity.

## Isolation and identification of *S. cerevisiae*

Dry bakery yeasts which were imported from different origns were collected from local markets, Which included; AY isolate from Angel yeast (Chinese origin), TY isolate from Town-instant (Chinese origin), YY isolate from Yuva yeast (Turkish origin), GY isolate from Gloripan (Egypt origin) and SY from Saf-instant (France origin). All isolates streaked on Sabouraud dextrose agar and incubated for 24 hrs at 30 ºC, then it examind under microscope and made conventional biochemical tests according to (Herrero *et al*., 1999; Lodder, 1974; Barnett *et al*., 1985).

## Cultivation of *S. cerevisiae*

Twenty Five ml of YEGP broth (10) g yeast extract, (20) g Glucose and (20) g Peptone in (1000) ml of distilled water) inoculated with a fresh culture of *S. cerevisiae* YY and incubated at 30 °C for 24 hrs. and further used as the seed culture for the production of biosurfactant (Dhivya , 2014).

Print ISSN: 2073-8854 & Online ISSN: 2311-6544

**Biosurfactant Production**

200 ml of YPEG broth medium pepared into 500 ml of erlenmyer flask. After cooling to 50 °C , 2 mL of seed culture was added to each container. The containers were incubated and shaken (125) rpm at (28-30)°C for 48 h

Use as crude biosurfactant (Farahnejad *et al*., 2004**).**

**Screening methods for biosurfactant**

**Oil-spreading test**

To apply the oil-spreading test, oil was layered over water in a petri plate and a drop of supernatant and pellet was added to the surface of oil. The diameter of the clear zone on the oil surface was measured in 2 replications for each isolate. A water drop was used as a negative control (Shoeb *et al*.,2015)

**Measurement of emulsification activity**

The emulsification index test was done to method described by (Shoeb *et al*.,2015) used to detect activity of biosurfactant, as follows: Emulsifying capacity of isolates was evaluated by an emulsification index (E24) for kerosene oil. To do so, 1.5 mL of Kerosene was added to 1.5 ml of supernatant and pellet in a test tube, which was vortexed at high speed for (2) min and allowed to stand for 24 h. The percentage of the emulsification index was calculated using the following equation.

E24 = Height of emulsion formed × 100/total height of solution.

**Isolation of biosurfactant**

The biosurfactant was extracted from *S. cerevisiae* by using three different treatments ( absolute ethanol , ammonium sulphate and boiling ). And use broth without any treatment .

Crude biosurfactant was prepared as previous and treated with following treatments:

First treatment ; 25 ml of previous culture was treated with five volume of absolute ethanol for 30 and 60 min in an ice bath , then centrifuged at 10000 rpm at 4ºC for 15min, then the pellet was taken and dissolved in an amount of PBS [(8) g NaCl, (0.2)g KCL, (1.44)g $Na_2HPO_4$ , (0.27)g $KH_2PO_4$ were dissolved in 800 ml of sterile distilled water .pH 7.4] after then assay the biosurfactant activity .

Second treatment ; 25 ml of culture was treated with heating to 100 c for 30 and 60 min , then centrifuged at 10000 rpm at 4ºC for 15min, then the supernatant was taken and test biosurfactant activity to determine the best time to heating.

Third treatment ; a specific weight of ammonium sulfate crystals was added to the (100 ml)of crude biosurfactant gradually in an ice bath with continuous stirring for 30 min to get saturation percentage of (0-25 , 25-50 , 50-75 and 75-100 %), then centrifuged at 10000 rpm at 4ºC for 15min, then the pellet was taken and dissolved in an amount of PBS . then dialyzed over night against the same buffer at 4 ºC. and assay biosurfactant activity to determine the best saturation percentage.

**Results and disscusion**

We obtained five isolates of *S. cerevisiae* from dry baker yeast where were blue color , oval to round with bud under microscope . After incubatation at 35c for 24 hrs, the colonies on Sabouraud agar were small, convex, regular margins and distinct odor. They were fermented to carbohydrates sources except (Lactose) because they don't have the enzyme lactase, Also It was produced ester odor on YPD agar , for While it can not hydrolysed the urea because they do not possed urease enzyme therefore the indicator color did not change (Lodder,1970).

Print ISSN: 2073-8854 & Online ISSN: 2311-6544

***Screening of S. cerevisiae for biosurfactant Production***

Five *S. cerevisiae* isolates were screened for cell-bound and excreted biosurfactant production by two methods; Surface activity by Oil spreading test and Emulsification index (E24).

Oil spreading assay is a reliable method to detect biosurfactant production by diverse microorganisms based on the ability of the biosurfactants present in the supernatant of isolate solutions capable of spreading the oil and producing a clear zone.This clearing zone on the oil surface correlates to surfactant activity, also called oil displacement

activity (Walter et al., 2010).

All isolates of *S. cerevisiae* were positive for the oil-spreading assay , but *S. cerevisiae* YY isolate showed the highest oil spreading activity (table-1).

**Table 1: The clear zone of biosurfactant on oil surface layer**

| Code of isolate | oil displacement diameter (mm) | |
|---|---|---|
| | Pellet of cells | Supernatant of cells |
| AY | 12 | 7 |
| TY | 8 | 5 |
| YY | 18 | 8 |
| GY | 9 | 6 |
| SY | 6 | 4 |

The diameter of the clearing zone formed by biosurfactant-containing solution has been shown to be directly proportional to the concentration of biosurfactant (Morikawa *et al*., 2000**)** . Results are displayed in Figure (1)

**Figar 1 : Oil spreading assay using boiling method**

Emulsification index (E24) assay is an indirect method used to screen biosurfactant production , where was different percent among *S. cereviasiae* isolates (Figure 2) , (table 2)

Print ISSN: 2073-8854 & Online ISSN: 2311-6544

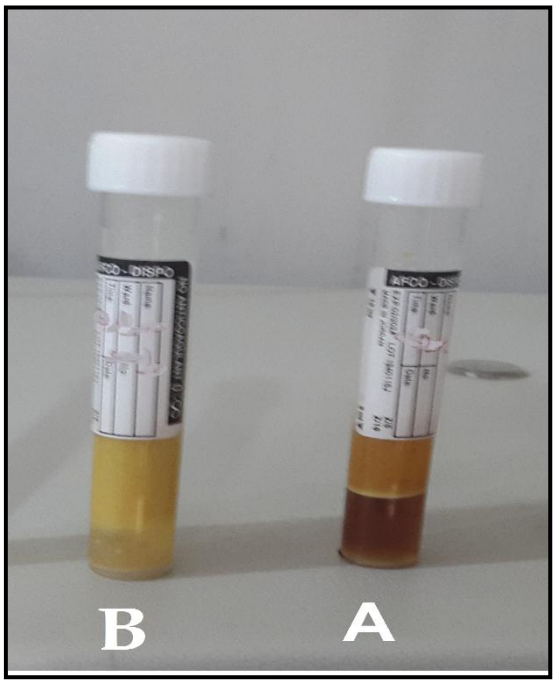


**Figure 2: Detection of biosurfactant activity by E24 assay , where**

**(A) Was represent Oil with culture not treated and (B) represent Oil with culture treated by boiling for 30 min.**

**Table 2: Percentage of emulsification index for biosurfactant**

| Code of isolate | Emulsification index % | |
|---|---|---|
| | Pellet of cells | Supernatant of cells |
| AY | 12.7 | 12.27 |
| TY | 8.18 | 7.72 |
| YY | 13 | 11.36 |
| GY | 9 | 9 |
| SY | 6.8 | 5.19 |

**Biosurfactant Production**

The selected biosurfactant-producing isolate *S. cerevisiae* (YY) was grown in yeast extract glucose pepton (YPEG) production medium, pH 5.6 incubated at 30$^{o}$C for 24 hrs in shaking incubator was centrifuged under refrigeration condition.

After incubation period , the flasks exhibited turbidity concomitant biomass and biosurfactant production.

**Isolation of biosurfactant**

In this present study , all treatments included precipitate the proteins .

We observed the boiling mehod for 30 min was the best treatment for biosurfactant extract from *S. cerevisiae* , That may be due to digestion of large portion of cell wall glucan and release the types of mannoprotein.

Print ISSN: 2073-8854 & Online ISSN: 2311-6544

Where many studies showed that the biosurfactant/ bioemulsifier was mannoprotein ( Alcantara *et al*., 2012 ; Dikit, *et al*., 2010 ; Walenckaa *et al*.,2007).
Alcantara, *et al*., (2011) reported that, the isolation of the biosurfactant by heat treatment for 5 min from *S. cerevisiae* 2031 cells effectively solubilised the extracellular biosurfactant.
Where The height emulsification index and oil displacement activity calculated as 81.08% and 20 mm , respectively

These result approach to The result of study of (Alcantara, *et al*., 2011), where declare that boiling of *S. cerevisiae* cells gave the highest E24 of 79.44%.

while boiling for 60 min effected on activity of biosurfactant where led to lowing it.

Absolute ethanol possibly react with component and product of the cell and led to damage it and lowing the activity of biosurfactant .

The biosurfactant was precipitated by ammonium sulphate with different percet according to saturation percents. the results showed that the best range of the biosurfactant was precipitated in the saturation range between (50 75%) . The E24 and surface activity was 42% and 10 mm respectively.

Ammonium sulfate is a salt, which is often used for the precipitation of proteins because of its high solubility (Burgess, 2009 ). The result was shown in table 3

**Table 3: The effect of different treatments on emulsification activity of biosurfactant/bioemulsifier from *S. cerevisiae***

| Treatments | **Emulsification index %** | **oil displacement diameter (mm)** |
|---|---|---|
| Boiling<br>For 30 min | 81.08 | 20 |
| For 60 min | 40.2 | 8 |
| Five folume of Absolute ethanol<br>30 min<br>60 min | 7<br>- | 2<br>- |
| Ammonium sulphate<br>0-25 %<br>25-50%<br>50-75%<br>75-100% | 5.7<br>11.3<br>42<br>13.2 | 2<br>3<br>6<br>3.5 |

Print ISSN: 2073-8854 & Online ISSN: 2311-6544

## Conclusion

Boiling method for 30 min for Isolation of biosurfactant from *S. cerevisiae* YY was best than other treatmens where activity of biosurfacant was 81.08 % and 20 mm by emulsification index and surface activity , respectively. Where these method was very easy and do not Consume tools and spend short time .

## References

**Alcantara** , V. A.; Pajares , I. G.; Simbahan , G. F. and Rubio , M. L. D. (2011). "Substrate Dependent Production and Isolation of an Extracellular Biosurfactant from *Saccharomyces cerevisiae* 2031 ". (2012, June ). **Philippine Journal of Science**. 141 (1): 13-24.

**Barnett** , J. A.; Payne, R. W. and Yarrow, D. (1985). Characteristic and Identification In : Yeast.2nd ed.,Cambridge University Press .pp.8-45.

**Burgess**, R. R. (2009). Deutscher, Richard R. Burgess and Murray P., ed. Methods in Enzymology. Guide to Protein Purification, 2nd Edition. 463, 331–342.

**Dhivya** , H.; Balaji , S.; Madhan , R. and Srv , A. (2014). Production of Amphiphilic Surfactant Molecule From Saccharomyces Cerevisiae Mtcc 181 And Its Protagonist In Nanovesicle Synthesis. (2014, November) **International Journal of Pharmaceutical Science Invention**. 3 (11):16-23.

**Dikit**, P.; Maneerat, s.; Musikasang, H. and H-kittikun, A. (2009). Emulsifier properties of the mannoprotein extract from yeast isolated.(October, 2010). **Science Asia.** 36 : 312–318.

**Farahnejad** , Z. J.; Rasaee, M.; Yadegari, H. and Moghadam, M. F. (2004). Purification and characteriazation of cell wall mannoproteins of *Candida albicans* using intact cell method. (2004, August). **Medical Journal of the Islamic Republic of Iran**. 18(2):167-172.

**Fracchia** , L.; Cavallo,M.; Martinotti, M.G.; and Banat, I.M. (2012). Biosurfactants and Bioemulsifiers Biomedical and Related Applications –Present Status and Future Potentials. (2012, 12 January). **Biomedical Science, Engineering and Technology**. 325-371.

**Herrero ,** M. B.; de Lamirande, E. and Gagnon, C. (1999). Nitric oxide regulates human sperm capacitation and protein-tyrosine phosphorylation in vitro. (1999, September ). **Biology of Reproduction**. 61: 575-58.

**Lodder,** J. (1970). The Yeast , A Taxonomic study , North-Holland.

**Luna** , J. M.; Rufino, R. D.; Sarubbo, L. A.; M. Rodrigues, L. R.; Teixeira, G, A. C. and Campos-Takaki, G. M. (2011). Evaluation Antimicrobial and Antiadhesive Properties of the Biosurfactant Lunasan Produced by Candida sphaerica UCP 0995. (2011, February). **Current Microbiology**. DOI 10.1007/s00284-011-9889-1.

**Morikawa ,** M.; Hirata , Y. and Imanaka , T. (2000). A study on the structure-function relationship of lipopeptide biosurfactants. (2000, Novomber). **Biochimica et *Biophysica* Acta**. 1488 (3): 211–218.

**Prabakaran** , M. and Sumathi, R. (2014). Studies on the biosurfactant producing pseudomonas aeuroginosa and its antimicrobial activity on selected human pathogens.(2014). **International Journal of Current Research in Chemistry and Pharmaceutical Sciences**. 1 (2) 69-78.

**Shoep** , E.; Ahmed, N.; Akhter, J.; Badar, U.; Siddiqui, K.; Ansari, F. A.; Waqar, M.; Imtiaz, S.; Akhtar, N.; Shaikh, Q. A.; Baig, R.; Butt, S.; Khan, S.; Khan, S.; Hussain, S.; Ahmed, B. and Ansari, M. A. (2014). Screening and characterization of biosurfactant-producing bacteria

isolated from the Arabian Sea coast of Karachi. (2015). **Turkish Journal of Biology**. 39: 210-216.

**Sridhar** , B.; Karthik, R.; Tamil , V. M. and Khanna, G. (2013). Production, Purification And Antimicrobial Activity Of Biosurfactants From *Saccharomyces Cerevisiae* And *Pseudomonas Aeruginosa. (2013, December).* **International Journal of Advance Research in Pharmaceuticals and Bioscience** .3(4), 1-7.

**Trindade** , J. R.; Freire , M. G.; Amaral , P.F.F; Coelho , M. A. Z.; Coutinho , J. A. B. and Marrucho , I. M. (2008). Aging mechanisms of oil-in-water emulsions based on a bioemulsifier produced by *Yarrowia lipolytica* . *(2008, July).* **Colloids and Surfaces A*: Physicochemical and Engineering Aspects*.** 324 ,149–154.

**Vidhya** , V.; Vidhya , A.; Gowri , B. V. and Arunadevi , S. (2015). Utilization of Synthetic Dairy Waste Water and Waste Oil for the Production of Sophorolipid from Starmerella bombicola MTCC 1910 and Testing its Antimicrobial Activity.(2015). **International Journal of Current Microbiology and Applied Sciences**. 4(12): 555-565.

**Walenckaa** , E.; Wieckowska-Szakiela ,M.; Rozalskab , S.; Sadowskaa , B. and Rozalskaa , B. (2007). A Surface-Active Agent from *Saccharomyces cerevisiae* Influences Staphylococcal Adhesion and Biofilm Development . (2007, November). http://www.znaturforsch.com .62, 433D438.

**Walter**, V., Syldatk, C., Hausmann, R. 2010. Screening Concepts for the isolation of biosurfactant producing microorganisms. ( **Journal of Bioscience and Technology**. 1: 120–126.

**Whitaker** , J. R. (1972). Principles of Enzymology for the Food Science. (ed. Oen, R. F. ) Marcel Dekker INC. New York.

**Willem** , E. (2012). Evaluation of the reduction of *Candida albicans* biofilm growth with biosurfactant AC7 in combination with antifungal drugs. Thesis. Department of Pharmaceutical Analysis .Ghent university. Belgium.