Nibras Nazar Mahmood /J. Pharm. Sci. & Res. Vol. 10(3), 2018, 481-486

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Effect of biosurfactants purified from *Saccharomyces cerevisiae* against *Corynebacterium urealyticum*

Nibras Nazar Mahmood
Mustansiriyah University, Department of Biology, College of Science, Baghdad, Iraq

Abstract:
Biosurfactant are amphiphilic compound have been progressively more attracting interest of the scientific community as promising candidates for the replacement of a number of synthetic surfactants. *Saccharomyces cerevisiae* (3) was isolated from imported dry yeast which were available in local market.extracted mannoproteins with emulsification properties from the cell walls of *S. cerevisiae* (3) cultivated in YPEG medium by three step to purification; in the present study, cell wall mannoproteins of intact yeast were purified using a simple treatment of yeast with mercaptoethanol and sodium dodecyl sulfate followed by ion exchange chromatography and by gel filteration chromatography. The pure mannoprotein was extracted display emulsion activity of 80% towards Kerosin oil as oil-in-water, while the surface activity was 37mm . The molecular weight of manoprotein was determined, where was 89.1 KD. the pure biosurfactant had higher inhibitor activity against *Corynebacterium urealyticum* , than partially purified where hieghst diameter of inhibition zone was (18mm).

INTRODUCTION :

S.cerevisiae is a model organism that is generally referred to as baker's yeast (Duina *et al.*, 2014). It was the first eukaryotic organism whose genome was fully sequenced in 1996,it became a promising model for many studies with its simple genome and short growth period (Yılmaz *et al.*,2012).Belong to the kingdom of Fungi, class; Ascomycetes, phylum *(Ascomycota*), family *Saccharomycetaceae* (Linder, 2012). *S. cerevisiae* is a single cell organism that contains a nucleus and other membrane organelles.(Dickinson and Schweizer, 2004).

Saccharomyces cerevisiae comes in the forefront of these microorganisms that have been fully exploited for benefit from it. (Mohamudha and Ayesha ,2010),these substances exhibit a variety of useful properties like, antimicrobial and antiadhesive agents (Johny, 2013), and could have some applications in the food and cosmetics industries. *S. cerevisiae* have ability to produce compounds called biosurfactant (Dikit *et al*, 2010).

Biosurfactants is biological surface active compounds produced by living cells microorganisms, mainly bacteria, fungi and yeasts (Płaza *et al.*, 2014**)**. They are produced on microbial cell surfaces or are extracellularly secreted (Salman*et al.*,2016**)**, which contains both hydrophobic and hydrophilic moieties (Vijayakumar *et al.*,2016) that confer the ability to accumulate between fluid phases and thus it is used in reduction of interfacial tension (Kalyani *et al.*, 2011).

Corynebacterium urealyticum (previously known as the coryneform CDC 97 group D2) , was first predictable to complex in human infections 80 years ago . It is Gram positive , slow growing ,lipophilic, multidrug resistant (Duztas *et al.*, 2006) .It is an opportunistic nosocomial pathogen causing acute cystitis , pyelonephritis , alkaline encrusted cystitis and may also cause bacterimia perefrential in patients with chronic urological diseases (Sorino and Tauch ,2008).

Colonies are characteristically pinpoint, whitish, smooth, convex, and non-hemolytic (Bernard *et al.*,2005). The common strains are greatly resistant to a large number of antibiotics, Including beta-lactams and macrolides although teicoplanin and vancomycin remain generally active against these isolates (Tauch *et al.*,2008).

MATERIAL AND METHOD:

Isolation and identification of *S. cerevisiae*

Dry bakery yeasts which were imported from different origns were collected from local markets. 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).

Cultivation of *S. cerevisiae*

inoculated fresh culture of *S. cerevisiae* (3) in twenty five ml of YEGP broth (10 g yeast extract, 20 g Glucose and 20 g Peptone in 1000 ml of distilled water). and incubated at 30 °C for 24 hrs. and further used as the seed culture for the production of biosurfactant (Dhivya.H1, 2014**).**

Biosurfactant Production

Biosurfactant production was performing according to Farahnejad *et al.*, (2004) with slight modification. One liter of YPEG broth medium was prepared, with divided it into two erlenmyer flask. After cooling to 50 °C, 2 mL of *S. cerevisiae* suspension was added to each container . The containers were incubated and shaken (125) rpm at (28-30)°C for 36 hrs .

Screening methods for biosurfactant

Applied 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

Explained method to emolsification index test by (Shoeb *et al.* 2015) used to detect activity of Mannoprotein as biosurfactant, as follows:emulsifying capacity of isolates was evaluated by an emulsification index (E24) for kerosene oil. 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.

Extraction of biosurfactant

Achieved according to (Farahnejad et al., 2004) with slight modifications.

Determination of protein concentration
Protein concentration was carried out using the Bradford method (1976).(Fig.1)

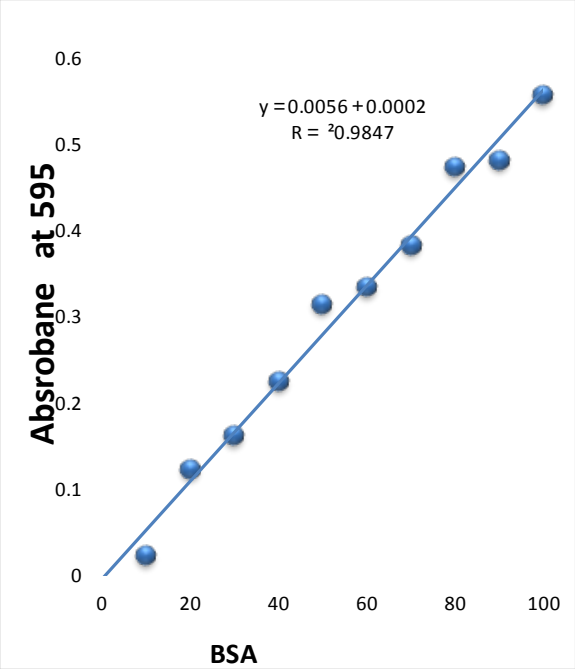

Figure (1): A standard curve of Bovine Serum Albumin for the determination of protein concentration.

Determination of carbohydrate concentration
Carbohydrate concentration was carried out using the Dubois *et al*. (1956) method (Fig.2)

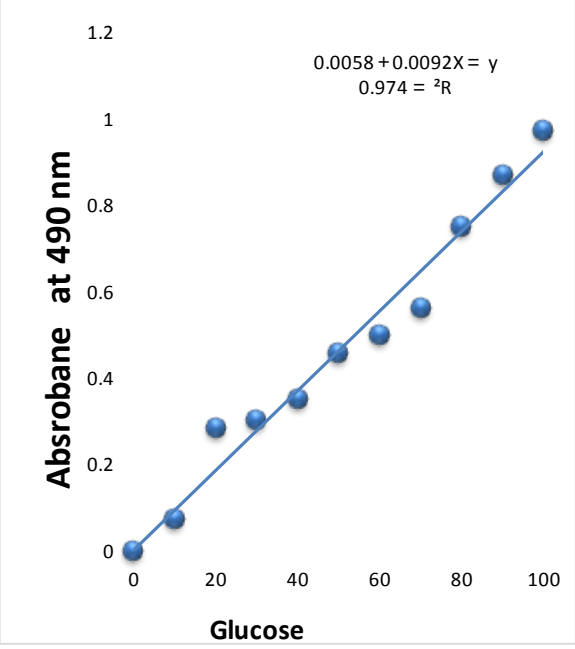

Figure (2): A standard curve of Glucose for the determinatio of carbohydrate concentration.

Purification of biosurfactant
Ion–exchange chromatography
Used column (1.5 × 8.5) cm of DEAE–cellulose to apply the concentrated material. Equilibrated with the PBS prepared according to (Sambrook *et al*,1989) . Washed column with the same buffer and eluted with (0.25,0.5 ,1) M of NaCl , at flow rate 1ml/min. Fractions (5ml) were assayed for absorbance at (280) nm and (490) nm for measurement protein and carbohydrate concentration. Then test the emulsification index for detect biosurfactant activity. The biosurfactan peak fractions were collected and concentrated with sucrose at 4ºC.

Gel filtration chromatography
Concentrated material from the prior purification step was applied to a column (1.5 × 60) cm of Sepharose–6B equilibrated with PBS, the column was eluted with the same buffer at flow rate (1ml/min) . The fraction (5) ml were collected and determine the protein and carbohydrate concentration. Then make tests of the emulsification activity and surface activity for these fractions. The biosurfactan peak fractions were pooled and concentrated with sucrose at 4ºC.

Determination of molecular weight
The method of gel filtration on a column Sepharos-6B was followed to estimate the molecular weight of the Mannoprotein, using a standard proteins by drawing the relationship between the logarithm of a standard protein molecular weight and the size of recovery size of Void (Ve/Vo),molecular weight was calculated as shown in the following steps:

1- Determination of the void volume of the column
Column preparation: The column sepharose- 6B with dimensions (1.5 × 80) cm, was used for purification of the glycoprotein; carry out its using PBS solution concentration of 0.1M, Void volume determined by using blue dextran to recovered parts the same budget buffer. Measured absorbance in separate parts (5) ml at a wavelength of 600 nm.

2- Determination elution volume for the standard protein
Gel filtration was carried out for four of standard proteins that was prepared(Segal,1976), absorption was measured at 280nm in separated volumes to determine elution volume (Vo) for each standard protein. The relationship between elution volume percentages was blotted for each standard protein to the elution volume of blue dextran (Ve/Vo) against molecular weight logarithm. This way helped measure enzymatic molecular weight.

Isolation and identification of *C. urelyticum*
Twenty-five isolates were collected from hospital belonging to the City of Medicine in Baghdad identified by using conventional method and vitek2 system (ANC) Card. (Salem *et al*.2015)

Antibiotic Susceptibility Test
Kirby-Bauer method was used described by Baron and Finegold (1990) to carry out the antibiogram test. pathogenic bacterial culture of (18-24) h was compared with the standard turbidily solution , this approximately equals to (1.5) cfu/ml. 0.1 ml of the culture was spread on the surface of Mueller-Hinton agar plates by curved glass rode, left to dry for 15 minutes at room temperature. The antibiotic discs were placed on the surface of inoculated medium and incubated at 37ºC for 24 h. Inhibition zones measured by a ruler and compared with the zones of inhibition determined by Clinical Laboratories of standards Institute (CLSI, 2015).

Effect of biosurfactant against *C. urelyticum in vitro:*
Mueller Hinton agar was prepared and poured in a sterile petriplate. The plates were allowed to solidify. Sterile filter paper disc (6) mm were Saturated with partial purified and pure of biosurfactant. 24 hrs. broth culture of *C. urelyticum* was swapped with sterile cotton swabs on Muller HintonThen, distribute the

discs into the plates. The plates were incubated at 37°C for 24 hours. After incubation period, the zone of inhibition was observed around the disc and it was measured (Cao *et al*., 2009) .

RESULTS AND DISCUSSION

Isolation and identification of *S. cerevisiae*

It was possible to get four isolates belonged to *S. cerevisiae* that isolated from dry bakery's yeast. All isolates were subjected to morphological and cultural tests as well as biochemical tests ,showed the belonging of the isolates mentioned to yeast(*S. cerevisiae*) by comparing these tests with scientific references to diagnosis yeast (Dabhole and Joishy,2005).

Screening methods for biosurfactant production

Four *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).

Measurement surface activity by Oil spreading test

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). oil spreading assay results were in corroboration with emulsification assay results. Strains found with positive drop collapse results were positive for oil spreading assay also. All isolates of S. cerevisiae were positive for the oil-spreading assay and *S. cerevisiae* isolates showed the highest oil spreading activity. Results showed in table (1).

Table 1: oil spreading activity by *S. cerevisiae* isolates

Code of isolate	Emulsification index %	
	Pellet of cells	Supernatant of cells
S. cerevisiae **(1)**	12.7	12.27
S. cerevisiae **(2)**	8.18	7.72
S. cerevisiae **(3)**	13	11.36
S. cerevisiae **(4)**	9	9

Observed the intracellular biosurfactant had higher surface activity than the extracellular biosurfactant. Method of oil spreading is rapid and easy to carry out, without need specialized equipment, and only required a small volume of sample (Shoep et al.,2015; Plaza et al., 2006). For all the studied isolates, the levels of cell-bound biosurfactant production were found to be higher than the excreted ones ((Joshi and shekhawat ,2014).

Emulsification index (E24)

Emulsification assay is an indirect method used to screen biosurfactant production get surface tension reductions between 9 mm and 13 mm using other *Lactobacillus* strains (Shoeb et al.,2015).Table (2).

Table2: Emulsification index percentag for biosurfactant

Code of isolate	oil displacement diameter (mm)	
	Pellet of cells	Supernatant of cells
S. cerevisiae **(1)**	12	7
S. cerevisiae **(2)**	8	5
S. cerevisiae **(3)**	18	8
S. cerevisiae **(4)**	9	6

Extraction and purification of biosurfactant

Extraction of the protein

Selected isolate of *Saccharomyces cerevisiae* (3) that produced biosurfactant, was grown in yeast extract glucose pepton(YPEG) production medium, pH 5.6 incubated at 30°C for 24 hrs in shaking incubator was centrifuged under refrigeration condition,after treatment with 2% Sodium dodecyl sulphate (SDS) and 5%ǀMercaptoethanol (ME) in 0.1M acetate buffer and the extracted used as partial purified biosurfactant . Biosurfactant activity by oil displacement and emulsification index was 20 mm and 45.4%respectively.

Ionic Exchange Chromatography

The method depending on the surface molecule charge, protein and the buffer conditions This is the most practical methods for protein purification, the protein will have net a positive or negative charge (Segel,1976). Ionic exchange chromatography patterns showed one protein peak in wash elution and three peaks in gradient elution. Only one peak among the gradient elution peaks represented biosurfactant activity (tubes 16-21).The biosurfactant activity appear in elution fractions (0.5 M of NaCl), that meant biosurfactant had negative charge enable it to bind with the resin of ion exchange which have positive charge.Result display in (Figure 3)

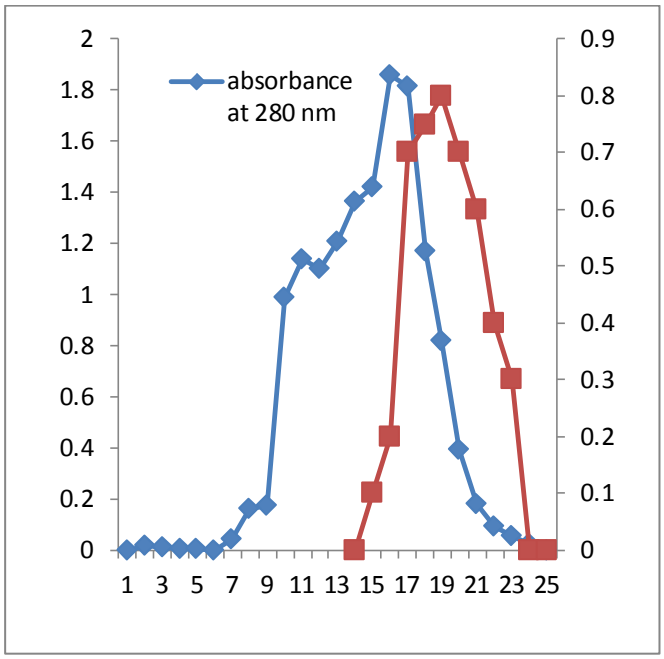

Figure 3: Ionic Exchange Chromatography for Mannoprotein from *Saccharomyces cerevisiae* through DEAE-Cellulose column (1.5 × 8.5) cm. The column was calibrated with 0.1M Phosphate Buffer Sline pH 7.4, flow rate 60ml/hrs and 5 ml fraction.

Gel filtration chromatography

Additional purification carried out by a gel filtration using Sepharose-6B. Protein fractions from DEAE-cellulose were pooled and passed through gel filtration column. The fractionation yielded three protein peaks as absorbance reading at 280nm and one peak as absorbance reading at 490nm (wave length). The second peak (fractionation tubes 17–22) had biosurfactant activity (35mm and 83.18% in oil displacement and emulsification index repectively), protein concentration (0.0535 mg/ml), while carbohydrate concentration was (0.08839 mg/ml) (Figure 4**)** and(Figure 5).

The Protein concentration of biosurfactantwas measured by bradforf method where was 0.0056 mg/ml. while carbohydrate concentration was 0.08839 mg/ml measured by Dubose method.

Determination of molecular weight

Estimated the molecular weight by gel filtration depending on the size of the separated molecules with their charge .Different methods of estimation may be used (Segal,1976). Sepharose-6B (1.5 × 60) cm was used for estimation the molecular weight of purified biosurfactant from *Saccharomyces cerevisiae* explained in figure (6). According to the logarithm molecular weight and elution volume/void volume (Ve/Vo). Standard curve the calculated (MW) of the biosurfactant found to be 89100 Dalton. (Table 3).

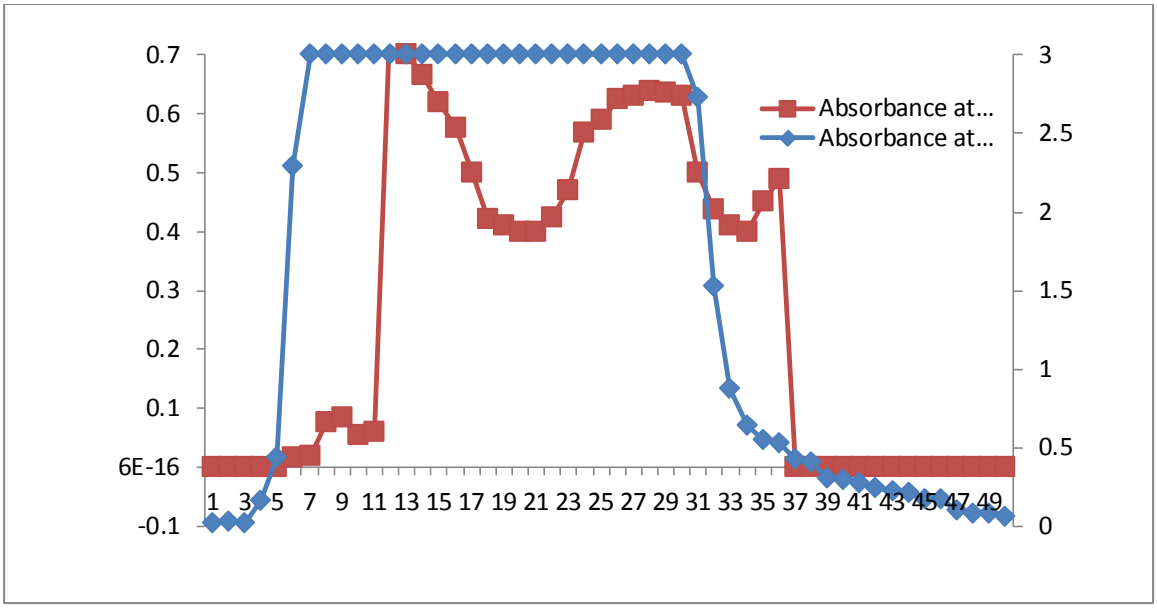

Figure 4 :Gel filtration chromatography (step 1) for purified biosurfactant from *Saccharomyces cerevisiae* by using Sepharose-6B column (1.5x80) cm. The column was calibrated with 0.1M PBS pH 7.2 ; flow rate 30 ml/hrs and 5 ml/fraction.

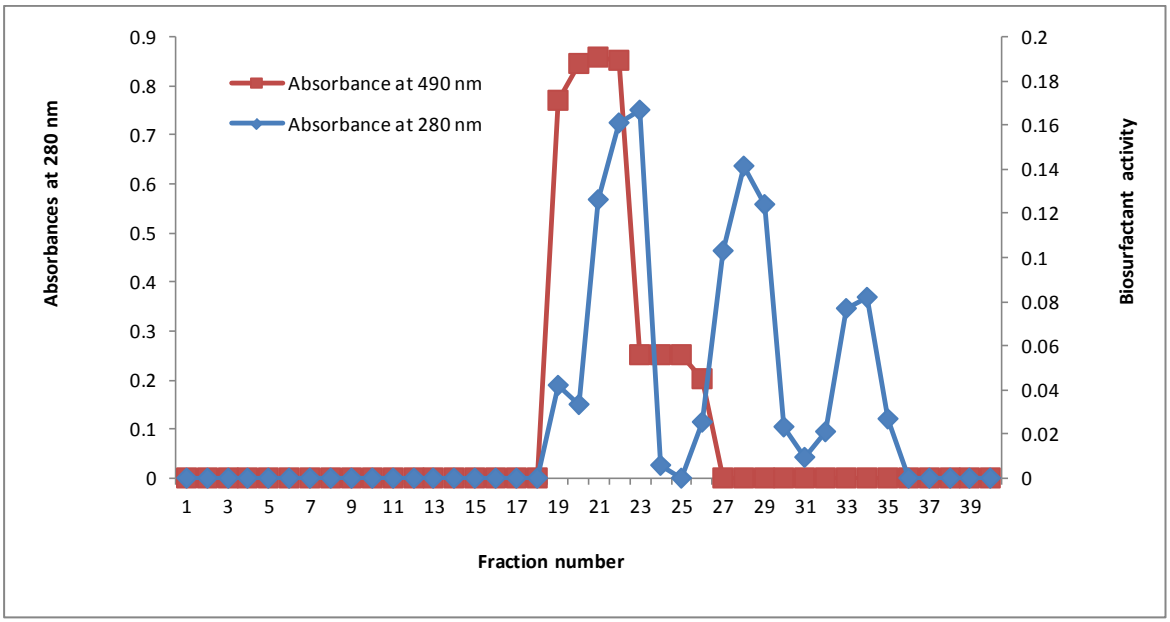

Figure 5 :Gel filtration chromatography (step 2) for purified biosurfactant from *Saccharomyces cerevisiae* by using Sepharose-6B column (1.5x80) cm. The column was calibrated with 0.1M PBS pH 7.2 ; flow rate 30 ml/hrs and 5 ml/fraction.

Table 3 Molecular weight of standard proteins

Standard protein and purified mannoprotein	**Ve/Vo**
Bovine Serum Albumin (232000D)	1.86
Casein (31000D)	2.15
Catalase (232000D)	1.365
Superoxidase dismutase (89000D)	1.798
Mannoprotein ((89100D)	1.66

Identification of *C. urelyticum*

Four isolates were used in the present study belong to (*C.urealyticum)* , were obtained from genital tract infection and identification by the vitek2 system (ANC) Card .

Antibiotic susceptibility Test

In current study the susceptibility is tested for Gram positive bacterial isolates were tested against (11) type of antibiotics; Gentamicin , Streptomycin , Cefotamixe , Ciprofloxacin , Penecillin G , Amoxicillin , Tetracycline , Rifampicin , Cloxacilline , Erythromycin and Chloramphincol . The results appears in the table (4) that presented various in the isolates resistance in this study against antibiotics uses . The results showed *C.urealyticum* isolates resistance to ;(Gentamycin , Rifampicin , Cloxacilline , Erythromycin and chloramphincol) while sensitive to other antibiotics uses in present. Antibiotic resistance is a problem of deep scientific concern both in hospital and community settings (Shaikh *et al.,* 2014). A principal mechanism for the spread of antibiotic resistance is by horizontal transfer of genetic material (Woodford, *et al.*2011). Resistance to beta-lactams , clindamycin , erythromycin , azithromycin , ciprofloxacin and Gentamicin was common among strains of *C.xerosis* and *C.minutissimum* (Coyle and Lipsky.,1990).

Table 4:Antibiotic sensitivity for *C.urealyticum*

Isolates No.	GN	S	CE	CIP	P	AX	TE	RA	CX	E	C
C. urelyticum (a)	R	S	S	S	S	S	S	R	S	R	R
C. urelyticum (b)	R	S	S	S	S	R	S	R	R	R	R
C. urelyticum (c)	R	S	S	R	S	S	S	R	R	R	R
C. urelyticum (d)	R	S	S	S	S	S	S	R	R	R	R

R:resistance , S:sensitive
GN:Gentamicin , **S**:Streptomycin , **CE**:Cephotamixe , **CIP**:Ciprofloxacin , **P**:Pencillin , **AX**:Amoxicillin , **TE**:Tetracycline , **RA**:Rifampicin , **CX**:Cloxacillin , **E**:Erythromycin , **C**:Chloramphincol.

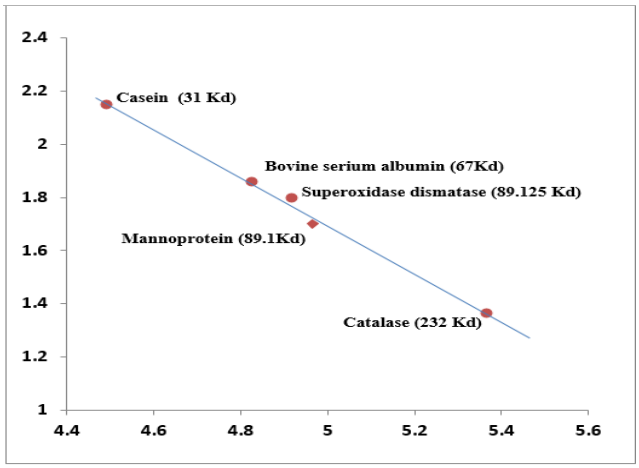

Figure 6: Standard curve to estimate molecular weight of Mannoprotein produced by *S. cerevisiae* using gel filtration by using Sepharose-6B.

Effect of biosurfactant against *C. urelyticum in vitro:*

Study the effect of partially purified and pure biosurfactant by using disc diffusion method against *C. urelyticum* isolates . The results showed in table (5) that pure biosurfactant had heighr inhibitory effect against all isolates under study .When the result reaveled that high diameter of inhibition zone by pure biosurfactant was (18mm) and (8mm) by partially purified (Table 5).

Table (5): Diameter of inhibition zone (mm) by partially purified and pure biosurfactant from S. cerevisiae 3 against *C. urelyticum*

Isolation No.	Partial purified (SDS and ME)	Pure biosurfactant
C. urelyticum (a)	5	11
C. urelyticum (b)	6	13
C. urelyticum (c)	8	18
C. urelyticum (d)	7	15

Our opinion in the difference in the sensitivity of the *C. urelyticum* clinical isolates of the biosurfactant which derived from *S. cerevisiae* yeast may be due to several factors that include ; the isolation site , containing genes which responsible for antimicrobial resistance additional to virulence factor like capsule , enzymes , cytotoxin in the form of an outer membrane protein named Omp38 . Walencka *et al.* (2007) showed that biosurfactant which produced from *S. cerevisiae* possesses surfactant activity, may be used as inhibitor of *Staphylococcus aureus* and *S. epidermidis* biofilm development. Johny ,(2013) cnfirmed that the purified external biosurfactant have inhibitory action against *Candida* and *Bacillus* .

CONCLUSION:

Ability of *S. cerevisiae* (3) to produce biosurfactant best than other straines used under this study. Pure biosurfactant extracted from baker's yeast and purifiued by Sepharose -6-B had anti bacterial activity against *C. urelyticum* than partially purified biosurfactant of the same yeast strain.

ACKNOWLEDGEMENTS

The current study supported by Mustansiriyah University (www.uomustansiriyah.edu.iq)-Baghdad/ Iraq.

REFERENCES

[1] **Baron, E. J.** and Finegold, S. M. (1990). Bailey and Scotts Diagnostic Microbiology. 8th (ed.). Mosby, Comp. Missouri.

[2] **Bernard, K**. (2005). Corynebacterium Species and Coryneforms: An Update on Taxonomy and Diseases Attributed to These Taxa. Clinical Microbiology Newsletter. 2005;27(2):9–18.

[3] **Bradford**, M. M. (1976). Arapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. Aral. Biochem. 72: 248-49.

[4] **Cao**, X.H., Z.Y. Liao, C.L. Wang, P. Cai, W.Y. Yang, M.F. Lu and G.W. Huang, 2009. Purification and and antitumor activity of a lipopeptidebiosurfactant produced by Bacillus natto TK-1. Biotechnology and Applied Biochemistry, 52: 97-106.

[5] **Clinical and Laboratory Standards Institute. (CLSI)**. (2015). Performance standard for antimicrobial susceptibility testing; Twenty-fifth informational supplement. M100-S25. 35(3): 56-58.

[6] **Coyle, M. B.,** and B. A. Lipsky. (1990). Coryneform bacteria in infectious diseases : clinical and laboratory aspects. Clin. Microbiol. Rev. 3:227-246.

[7] **Dabhole ,**M. P. and Joishy, K. N.(2005). Production and effect of killer toxin by *Saccharomyces cerevisiae* and *Pichia kluyveri* on sensitive yeasts and fungal pathogens. Indian Journal of Biotechnology .,4:290-292.

[8] **Dhivya**.H,;Balaji,S.; Madhan.Rand.Srv, A. (2014). Production of amphiphilic surfactant molecule from Saccharomyces cerevisiae Mtcc 181 and its protagonist in nanovesicle synthesis. *International Journal of Pharmaceutical Science. 11:16-23.*

[9] **Dickinson** and Schweizer. (2004). The metabolism and molecular physiology of Saccharomyces cerevisiae, 2nd edition

[10] **Dikit**, P.; Maneerat,s.; Musikasang,H. and H-kittikun, A.(2010). Emulsifier properties of the mannoprotein extract from yeast isolated.*ScienceAsia*36 : 312–318.

[11] **Dubois**, N.; Gilles, K.A.; Hamilton, J.K.; Rebers, P.A.; Smith, F.(1956). Colorimetric method for detection of sugars and relatedsubstances. Anal. Chem. 28: 350-356.

[12] **Duina**, A.A.; Miller, M.E.and Keeney, J.B.(2014). Budding Yeast for Budding Geneticists: A Primer on the *Saccharomyces cerevisiae* Model System. *Genetics*, Vol. 197, 33–48.

[13] **Duztas, MN.;** Erdem, H.; Pahsa, A.; Emekdas, G. and Avci, A.(2006) Epidemiology of Dworkin M, Falkow S. The Prokaryotes: Vol. 4: Bacteria: Firmicutes, *Cyanobacteria*. 3rd ed. Springer; 2006. 5p.

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

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

[16] **Johny,**J.M.(2013). Inhibitory effect of biosurfactant purified from probiotic yeast against biofilm producers. *Journal Of Environmental Science, Toxicology And Food Technology.6(1) : 51-55.*

[17] **Joshi** , P. A. and Shekhawat, D. B. (2014). Screening and isolation of biosurfactant producing bacteria from petroleum contaminated soil. *European Journal of Experimental Biology*. 4(4):164-169.

[18] **Kalyani**,R.; Bishwambhar, M.; and Suneetha, M. (2011). Recent Potential usage of surfactant from microbial origin in pharmaceutical and biomedical respective arena : a perspective .International reaserch journal of pharmacy.2 (8): 11-15.

[19] **Linder**, T.(2012). Genomics of alternative sulfur utilization in ascomycetous yeasts. *Microbiology*. 158, 2585–2597.

[20] **Mohamudha ,**P. R and Ayesha B. J. (2010). Production and effect of killer toxin by *Saccharomyces cerevisiae***.** International Journal of Pharmaceutical Sciences Review and Research.3(1):127-129.

[21] **Plaza** , G. H.; Chojniak, J. and Banat, I. M. (2014). Biosurfactant mediated biosynthesis of selected metallic nanoparticles. *International Journal of Molecular Sciences*.*15*,13720-13737.

[22] **Salman** , J. A.; Al Marjani1,M, F Abdul Ghani, Z. S. (2016). Inhibition of Cancer Cells Line by Biosurfactant Produced from *Leuconostoc mesenteroides* ssp *cremoris*. Iraqi Center for Cancer and Medical Genetics Researches. 3(2):121-125.

[23] **Sambrook ,**J.; Fritsch, E. F. and Maniatis, T. (1989). Molecular cloning: A laboratory Manual. 2nd ed. P.A. 12 Cold spring Harbor Laboratory press. Cold sipring Harbor, New York. P: 68.

[24] **Seagel**, I. H. (1976). Biochemical Calculations, 2nd ed. John and Sons. Inc. NewYork.

[25] **Shaikh, S.;** Fatima, J. ;Shakil , S.; Mohd, S.; Rizvi,D and Kamal, M.(2014). Antibiotic resistance and extended spectrum beta-lactamases: Types, epidemiology and treatment. SAUDI. J .BIOL. SCI.,(22): 90–101.

[26] **Shoeb**,E.; Ahmed,N.; Akhater,J.; Badar,U.; Siddiqui,K.; Ansari,F.A.; Wqar, M.; Imtiaz ,S.; Akhatar,N.; Shaikh ,Q.A.; Baig ,R.; Butt,S.; Khan, s.; Khan, s.; Husain,S.; Ahmed, B. and ANSARI,M.A. (2015).*Turk J Biol*.39: 210-216.

[27] **Soriano, F.** and Tauch, A**.(2008)** Microbiological and Clinical Features of Corynebacterium urealyticum: Urinary Tract Stones and Genomics as The Rosetta Stone. Clin Microbiol Infect. 2008;14(7):632–643.

[28] **Tauch, A**.; Trost, E. and Tilker, A. *et al. (*2008) The lifestyle of Corynebacterium urealyticum Derived from its Complete Genome Sequence Established by Pyrosequencing. J Biotechnol. 2008;136(1–2):11–21.

[29] **Vijayakumar**, V.; Rajenderan, S.; Laishram, S.; Anandan, S Balaji1, V. and Biswas, I. (2016). Biofilm formation and motility depend on the nature of the *Acinetobacter baumannii* clinical isolates. *Frontiers in Public Health*. 4 (105):1-9.

[30] **Walencka,E**.: Wieckowska-Szakiel ,M.:Rozalska,S.:Sadoweska,B.and Rozalska,B.(2007). Asurface-active agent from *Saccharomyces cerevisiae* influences staphylococcal adhesion and biofilm development . Z.Naturfrosch C.62(5-6): 433-8.

[31] **Woodford, N.;** Turton, J.F. and Livermore, D.M. (2011). Multi resistant gram negative bacteria: the role of high-risk clones in the dissemination of antibiotic resistance. FEMS Microbiol. Rev.(35): 736–755.

[32] **Yılmaz**, R.; Akça,O.; Baloğlu, M.C.; Öz, M.T.; Öktem, H.A and Yücel, M.(2012). Optimization of yeast (*Saccharomyces cerevisiae*) RNA isolation method for real-time quantitative PCR and microarray analysis. African Journal of Biotechnology Vol. 11(5), pp. 1046-1053.