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Antimicrobial Activity of a Bioemulsifier Produced by *Saccharomyces cerevisiae*

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Abstract

This study was designed to evaluate the ability of bioemulsifier to inhibit the growth of some pathogenic microorganisms. Different Iraqi source of *Saccharomyces cerevisiae* were tested for their ability to produce bioemulsifier. The production of bioemulsifier was detected by determination of emulsification index (E24%), extracted and purified, the antimicrobial activity of purified bioemulsifier was tested against some pathogenic microorganisms. Results revealed that bioemulsifier reduced the growth of bacteria (*Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Bacillus subtilis*).

Introduction

Yeasts are fungi, whose common characteristics are predominant or permanent unicellular state. Yeasts are unicellular eukaryotic fungi with completely different properties than those of bacteria, which are Prokaryotes. For example, yeasts have a resistant to antibiotics, sulfamides

and other anti-bacterial agents. This resistance is genetically and natural, and not liable to be modified or transmitted to other microbes. Moreover, yeast particle size ($5 \times 10 \mu\text{m}$) is also significantly higher than bacteria size ($0.5 \times 5 \mu\text{m}$). The main method of yeast reproduction is

primarily by budding, and occasionally by fission, and these do not form spores in or on a fruiting body. Among yeast, *S. cerevisiae* is of industrially important due to its ability to convert sugars (i.e., glucose, maltose) into ethanol and carbon dioxide (baking, brewing, distillery, liquid fuel industries). *S. cerevisiae* breaks down glucose through aerobic respiration in presence of oxygen. If oxygen is absent, the yeast will then go through anaerobic fermentation. Bioemulsifier are a heterogeneous group of surface active molecules produced by microorganisms, which either adhere to cell surface or are excreted extracellularly in the growth medium. These molecules reduce surface tension in both aqueous solutions and hydrocarbon mixtures Mulligan (2005). Two closely related organisms that share the same species and same genus can produce different types of bioemulsifier isoforms with different physicochemical properties which help the microorganism to survive Ron & Rosenberg (2001). The hydrophilic moiety is comprised of acid, peptide cations or anions, mono or polysaccharides while hydrophobic moiety can be unsaturated or saturated chains of fatty acids Maneerat & Dixit (2007). Mannoprotein has been shown to be an effective bioemulsifier. The presence of hydrophilic mannose polymers covalently attached to the protein backbone provides the mannoprotein with the amphiphilic structure common to surface active agents and effective emulsifiers Cameron *et al.* (1988). In the yeast, *Saccharomyces cerevisiae*, the cell

wall contains β (133)-D-glucan, β (136)-D-glucan, chitin, and mannoprotein Cabib and Roberts (1982). The polysaccharides appear to have a structural function, whereas the mannoprotein may act as “filler” and are important for the permeability of the cell wall (Zlotnik *et al.*, 1984). β –glucan has attracted attention because of its bioactive and medicinal properties as immune stimulating (Vetvicka *et al.*, 2008), antimicrobial, anti-infective, antiviral, wound-healing (Bohn and BeMiller,1995). β –glucan is known as a potent immunostimulant and has significant augmenting effects on the host defense system (Yadomae and Ohno,1996). Besides, β –glucan is known to process antimicrobial and antitumor activities by enhancing host immune function, and activating macrophages, neutrophils and NK cells by binding to the β –glucan receptor on these cells (Bohn and BeMiller,1995). β –glucan is a promising candidate as an immune – stimulatory agent for immune – compromised patients or those who are infected by multidrug-resistant bacteria (Yadomae and Ohno, 1996).

2. Material and methods

2.1 Yeast Collection

The sample were collected from different Eco sources like fruits, soil, juices, and fermented milk. The samples were collected and formed in a sterile glass tubes and compressed fifty grams/ml of material were enough to fill a universal tube, the tube was sealed in a way that permit gas under pressure to escape and to prevent the entry

of oxygen. Tubes were opened and the collected samples placed into Flasks containing sterile water which were vigorously Shaken, then. 1 ml was taken from these suspensions and then completed with 9 ml from D.W and transferred loop full by a sterile loop to the Yeast Malt Extract agar by streaking and incubated aerobically for (48) hrs. at (30) °C. (Deepa *et al.*, 2015).

2.2 Production of Bioemulsifier from *Saccharomyces cerevisiae*:

Bioemulsifier production were carried out in 250 mL Erlenmeyer flasks containing 50 ml of Cooper and Paddock's medium. One ml of the seed culture was added and the flasks were incubated at ambient temperature with shaking for 4 days. Cells were harvested by centrifugation at 3000 rpm for 10 min. (Alcantara *et al.*, 2014).

2.3 Extraction and Purification of Bioemulsifier from *Saccharomyces cerevisiae*

The extraction and production was carried out at an optimized condition for 48 hours and the bacterial cells were removed by centrifugation at 10,000 rpm for 20 min under cooling condition. Bioemulsifier extraction and solubilization was done by boiling (100°C) *S. cerevisiae* cells for 30 min in water, 0.067M phosphate buffer (PB) and 0.067M PB with 0.02M Na₂S₂O₅. The cells were then centrifuged at 3,000 rpm for 10 min and the supernatant was assayed for emulsification activity. After extraction process,

the precipitate was obtained. This precipitate was dissolved in maximum 3ml of chilled sterile distilled water and dialyzed extensively against sterile distilled water at room temperature for 48° C. Distilled water was changed after 12h. (Cameron *et al.*, 1988).

2.4 Determination of Emulsification Index (E24%)

The material to be tested was dissolved in 4 ml of distilled water in a test tube, 6 ml of kerosene (or another water-immiscible liquid) was added, and the tube was vortexed to homogeneity. After 1 h the proportion of kerosene emulsified was compared with the total volume of kerosene added. This parameter was known as the percentage of the kerosene phase that was emulsified. The content of kerosene in the emulsion was calculated by dividing the volume of kerosene in the emulsion phase by the total volume of the emulsion. In the absence of an emulsifying agent, emulsions generated by vigorous mixing separated completely within 1 h (Abouseoud et al.2008).

2.5 SDS-PAGE for Molecular Weight Determination

The molecular mass of the purified BE was estimated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) following the procedure of Laemmli (1970) using a minigel system (mini-Protean II dual slab cell; Bio-Rad). Samples (10 µL) were separated in 10% polyacrylamide slab gels, with a 4% acrylamide stacking gel, at a constant voltage of 100 V for 30 min. After electrophoresis, the gel

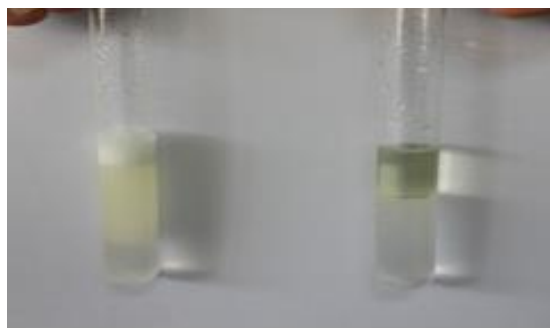
was stained with Coomassie Blue R-250 following standard procedure. Molecular weight markers were used to determine the molecular mass.

2.6 Antimicrobial activity of Bioemulsifier (Agar well Diffusion Method technique Perez *et al.* (1990))

The extract was tested for antibacterial activity by standard agar well diffusion method against pathogenic bacteria *E. coli* and *S.aureus*, *S. epidermidis*, *P. aeruginosa*, *B. cereus*, *B.subtilis*. The pure culture of bacterial pathogens was sub cultured on nutrient broth. 20ml of nutrient agar were poured into the Petri plates. Wells of 6mm diameter were made on nutrient agar using gel puncture. Culture was swabbed uniformly using sterile cotton swabs, and then 50µl of purified bioemulsifier solution was loaded into the wells. After incubation at 37°C for 24 hours, the different levels of zone of inhibition were measured.

3. Results

An initial screening of bioemulsifier producing isolates were grown in Yeast extract broth supplemented with oils and then tested for qualitative drop collapse and (E24%). Only one yeast isolates were showed maximum bioemulsifier activity and was selected as potent strain and used for further study.



Emulsification Index

3.1 Screening of Bioemulsifier

Bioemulsifier production is growth associated. The yeast strain of *Saccharomyces cerevisiae* produced bioemulsifier when it was grown in Yeast extract broth containing cooked oil. The highest emulsification index (E24%) value on the sunflower oil (75. %) followed by olive oil and corn oil (70%). (Figure-1) The minimum bioemulsifier was produced from *Saccharomyces cerevisiae* in the presence of soya oil, the results of drop collapse in microtiter plate for *Saccharomyces cerevisiae* showed a large spread in sunflower oil for the droplet in oil displacement, these isolates were exhibited oil displacement activity. Maximum oil displacement was obtained in sunflower oil (1.8mm) (figure-2).

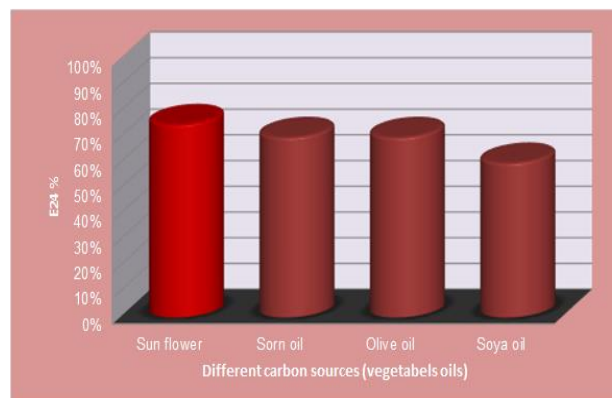


Figure 1: Effect of different Carbon Sources (vegetable oils) on bioemulsifier production



oil displacement.

Figure 2: (Screening Methods of Bioemulsifier Produced by *Saccharomyces cerevisiae*).

The extraction of *Saccharomyces cerevisiae* using Na₂S₂O₅ is a agent known to increase bioemulsifier yield and prevent oxidation. Heat extraction solubilizes the mannoproteins in the outer layer of the cell wall. After extraction of component can be further purified by using dialysis method. The Protein concentration of bioemulsifier was measured by Lowry method where was 3 mg/ml. while carbohydrate concentration was 1.7 mg/ml measured by Dubois method.

3.2 SDS PAGE Gel electrophoresis

The purified enzyme was resolved on a SDS-PAGE (5% stacking and 12% separating gel) found to be a homogenous monomeric protein as evident by a single band corresponding to (60) kDa for *S.cerevisia* on SDS-PAGE relative to the standard molecular weight markers, it was single band as shown in (figure-3) , the single band in dark smear refer to the bioemulsifier protein .

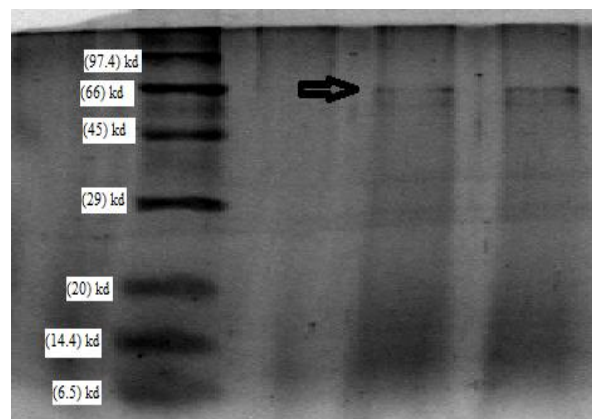


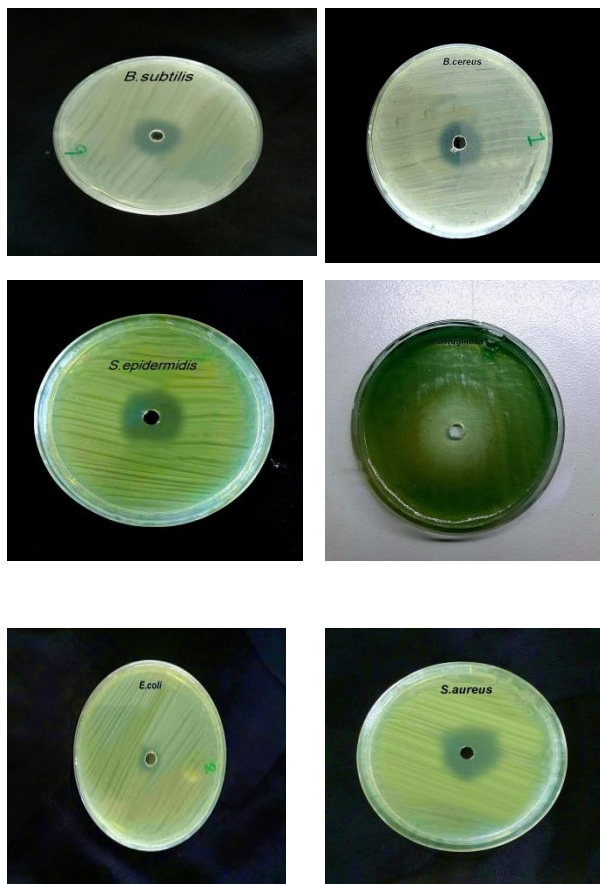
Figure 3: (SDS PAGE gel electrophoresis of Bioemulsifier from *S.cerevisia*).

3.3 Antimicrobial activity of Bioemulsifier (Agar well Diffusion Method)

The antimicrobial activity of purified bioemulsifier were investigated by agar well diffusion method against *E coli*, *S aureus*, *P.aeruginosa* pathogen *B.subtilis* *B.cereus* *S. epidermidis* (figure 4). The maximum zone of inhibition was obtained in bioemulsifier from *Saccharomyces cerevisiae* produce the zone of (25mm) against, *P. aeruginosa*. The minimum zone of inhibition were obtained in bioemulsifier from *Saccharomyces cerevisiae* produce (10mm) against *Escherchia coli* while against, *Staphylococcus aureus* and *S. epidermidis* was (18mm), it showed inhibitory activity against *B.cereus* (17mm) and (14mm) against *B.subtilis* so hereby concluded that the maximum zone were obtained in synergistic study were shown in (Table - 1).

Table 1: (Antimicrobial activity of purified Bioemulsifier)

Bacteria	Inhibition zone diameter (mm)	Control normal saline
<i>Pseudomonas aeruginosa</i>	25	No inhibition
<i>Staphylococcus epidermidis</i>	18	No inhibition
<i>Staphylococcus aureus</i>	18	No inhibition
<i>Bacillus cereus</i>	17	No inhibition
<i>Bacillus subtilis</i>	14	No inhibition
<i>Escherichia coli</i>	10	No inhibition

**Figure 4: (Antimicrobial activity of *S. cerevisiae* Bioemulsifier)**

4. Discussion

4.1 Screening of Bioemulsifier

As showed in the result of bioemulsifier production Different type of vegetable oils on bioemulsifier Production were used and the yeast strain of *Saccharomyces cerevisiae* produced bioemulsifier when it was grown in Cooper and Paddock's medium broth for 4 days at (30) °C the highest emulsification index (E24%) value on the sunflower oil (75% E24) followed by olive oil and corn oil (70% E24). The minimum bioemulsifier was produced from *Saccharomyces cerevisiae* G11 in the presence of soya oil, the results of drop collapse in microtiter plate for *Saccharomyces cerevisiae* showed a large spread in sunflower oil for the droplet in oil displacement, these isolates were exhibited oil displacement activity. Maximum oil displacement was obtained in sunflower oil (18mm). Javed Maniyar *et al.*(2011) revealed that the bioemulsifier production from *Streptomyces* sp.S22 was induced by sunflower oil and their bioemulsifier activity increased from 145 EU/ml to 320 EU/ml.(Mbawala and Mouafo, 2012) showed that the eight strains of *Lactobacillus spp.* have showed a positive result in emulsification test. The highest potential of emulsifying activity (% E24) of 56.80 was obtained from the fermentation broth of the TM1 isolate. (Jyotsna and Dharendra, 2014) observed that the *P. fluorescens* showed highest oil displacement for soybean oil (2.5mm) followed by 2mm for mustard oil.

4.2 SDS PAGE Gel electrophoresis

The result in this study showed that protein as evident by a single band corresponding to (55-60) kDa for *S.cerevisia* on SDS-PAGE relative to the standard molecular weight markers, Matthew Ilori & Dan-Israel Amund (2001) reported that the purified bioemulsifier of *P. aeruginosa* subjected to SDS PAGE. A single SDS - PAGE band was obtained from the purified protein complex preparation. The apparent molecular weight was estimated as 14,322 Da. (Alcantara et al, 2014) reported that weight of the bioemulsifier from *S. cerevisiae* was estimated to be a high molecular weight biosurfactant and its high emulsification activity and stability.

4.3 Antimicrobial activity of Bioemulsifier

The study also clearly demonstrated the antimicrobial effect of the bioemulsifier against deferent bacteria like against *E coli*, *S aureus*, *P.aeruginosa* pathogen *B.subtilis* *B.cereus* *S. epidermidis* and it give high inhibition activity against them , Abalos *et al.*(2001) reported that the rhamnolipid mixture obtained from *P. aeruginosa* showed inhibitory activity against the bacteria (*Escherichia coli*, *Micrococcus luteus* and *Alcaligenes faecalis*, *Serratia marcescens*, *Mycobacterium phlei* and *Staphylococcus epidermidis*) and fungi (*Aspergillus niger*, *Chaetonium globosum*, *Enicillium crysogenum*, *Aureobasidium pullulans*, *Botrytis cinerea* and *Rhizoctonia solani* in various concentrations. It is suggested to use this compound or Biosurfactants in pharmaceutical and cosmetics for dermal and other applications. because it has lethal effect on

other microorganism especially pathogenic bacteria.

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